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FILE COVERS 1907 - 19 Mar 2002  VOL 136 ISS 12
FILE LAST UPDATED: 18 Mar 2002  (20020318/ED)

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Numbers with the P indicator executed between 12/27/01 and 1/23/02 may
be incomplete.  See the NEWS message on this topic for more information.

=> d stat que
L1          194 SEA FILE=REGISTRY FLAA?
L2           57 SEA FILE=REGISTRY P37/BI
L4            1 SEA FILE=REGISTRY "T7 NA"/CN
L8           16 SEA FILE=REGISTRY (BORELLI/BI OR BORELLIA/BI)
L9         3726 SEA FILE=REGISTRY (BURGDORFBURGDORFERI/BI OR BURGDORFERI/BI OR
                BURGDORFERIA/BI)
L10         276 SEA FILE=HCAPLUS L1 OR FLAA
L11         288 SEA FILE=HCAPLUS L2 OR P37
L12         206 SEA FILE=HCAPLUS L4 OR T7(5A)GENE?(5A)10
L13        1789 SEA FILE=HCAPLUS L8 OR L9 OR BORELL? OR BURGDORF?
L14        2162 SEA FILE=HCAPLUS L13 OR LYME?
L15          22 SEA FILE=HCAPLUS (L10 OR L11 OR L12) AND L14


=> d ibib abs hitrn l15 1-22

L15  ANSWER 1 OF 22  HCAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:       2001:871464  HCAPLUS
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TITLE: Recombinant flagellin A proteins from Borrelia **burgdorferi** sensu stricto, B. afzelii, and B. garinii in serodiagnosis of **Lyme** borreliosis

AUTHOR(S): Panelius, Jaana; Lahdenne, Pekka; Saxen, Harri; Heikkila, Tero; Seppala, Ilkka

CORPORATE SOURCE: Haartman Institute, Department of Bacteriology and Immunology, University of Helsinki, Helsinki, FIN-00014, Finland

SOURCE: J. Clin. Microbiol. (2001), 39(11), 4013-4019
CODEN: JCMIDW; ISSN: 0095-1137

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Genes for flagellin A (**FlaA**) proteins from European borrelial strains of Borrelia **burgdorferi** sensu stricto, B. afzelii, and B. garinii were cloned and sequenced. An identity of 92 to 93% was obsd. in the **flaA** sequences of the different species. Polyhistidine-tagged recombinant **FlaA** (rFlaA) proteins were produced in Escherichia coli and used as antigens in Western blotting (WB) and ELISA (ELISA). In IgG (IgG) WB, 71% (10 of 14) of the sera from neuroborreliosis and 86% (12 of 14) of those from **Lyme** arthritis patients reacted with one to three rFlaAs. In IgG ELISA, 74% (14 of 19) and 79% (15 of 19) of patients with neuroborreliosis and arthritis, resp., were pos. The immunoreactivity in local European patient sera was stronger against rFlaA from B. garinii and B. afzelii than against rFlaA from B. **burgdorferi** sensu stricto. Neither IgG nor IgM ELISA was sensitive in the serodiagnosis of erythema migrans. Serum samples from patients with syphilis and systemic lupus erythematosus showed mild cross-reactivity in IgG tests. Sera from Yersinia enterocolitica or beta-hemolytic Streptococcus infections showed only occasional responses. With IgM ELISA, 58% (11 of 19) and 37% (7 of 19) of patients with neuroborreliosis and arthritis, resp., were pos. Cross-reactive antibodies to **FlaA**, esp. in serum samples from patients with rheumatoid factor positivity and Epstein-Barr virus infection, reduced the specificity of IgM serodiagnosis. Therefore, rFlaA seems to have a limited role for IgM serodiagnosis, yet rFlaA might be useful in the IgG serodiagnosis of disseminated **Lyme** borreliosis.

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 2 OF 22 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:801168 HCAPLUS

TITLE: Reactivity of dog sera to whole-cell or recombinant antigens of Borrelia **burgdorferi** by ELISA and immunoblot analysis

AUTHOR(S): Magnarelli, Louis A.; Levy, Steven A.; Ijdo, Jacob W.; Wu, Caiyun; Padula, Steven J.; Fikrig, Erol

CORPORATE SOURCE: Department of Entomology, Connecticut Agricultural Experiment Station, New Haven, CT, 06504, USA

SOURCE: J. Med. Microbiol. (2001), 50(10), 889-895
CODEN: JMMIAV; ISSN: 0022-2615

PUBLISHER: Lippincott Williams & Wilkins

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Enzyme-linked immunosorbent assays (ELISAs) with sep. prepns. of 10 purified recombinant antigens of Borrelia **burgdorferi** sensu stricto were used to test sera from 36 dogs not vaccinated with whole cells of this agent and from five dogs vaccinated with whole-cell B. **burgdorferi** bacteria. All dogs lived in tick-infested areas of Connecticut and south-eastern New York state, USA. The non-vaccinated dogs had limb or joint disorder, lameness and fever during the period 1984-1991 and had antibodies to B. **burgdorferi**, as detd. by a polyvalent ELISA with whole-cell antigen. In re-analyses of sera for total Igs in ELISAs with recombinant antigens, reactions were most frequently recorded when outer-surface protein (Osp) F, protein (p)35, **p37**, p39 and p-41G (a flagellin component) were tested sep. Western immunoblots of a subset of 16 sera, pos. by ELISA with whole-cell antigen and representing a range of antibody titers (640-40 960), verified immune responses to these or other lysed whole-cell antigens. Sera from vaccinated dogs contained antibodies to OspA, OspB, p22, **p37** and p41-G. Therefore, serol. reactions to OspF, p35 and p39 were the most important indicators of natural exposure to B. **burgdorferi**. Serum reactivities to these recombinant antigens in ELISAs can be used to help identify possible natural infections of canine borreliosis in dogs not vaccinated with whole-cell B. **burgdorferi** and to provide information on the geog. distribution of this bacterium.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 3 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:780618 HCAPLUS
DOCUMENT NUMBER: 135:343267
TITLE: Vaccines for protection against Borrelia **burgdorferi**
INVENTOR(S): Exner, Maurice M.; Lovett, Michael A.; Blanco, David R.
PATENT ASSIGNEE(S): Regents of the University of California, USA
SOURCE: PCT Int. Appl., 41 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001078650	A2	20011025	WO 2001-US11243	20010405

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2000-548947 A 20000413
AB This invention relates to the development of a vaccine for generating

immunoprotection against Borrelia **burgdorferi**, the causal agent of **Lyme** disease. Specifically, the invention relates to vaccines comprising an Oms66 polypeptide.

IT **370969-12-3**, Porin (Borrelia **burgdorferi** gene oms66)
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; vaccines comprising Oms66 polypeptide for protection against Borrelia **burgdorferi**)

L15 ANSWER 4 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:911291 HCAPLUS
DOCUMENT NUMBER: 134:70362
TITLE: Combined decorin binding protein and outer surface protein compositions and methods of use
INVENTOR(S): Hanson, Mark S.; Patel, Nita K.; Cassatt, David R.
PATENT ASSIGNEE(S): Medimmune, Inc., USA
SOURCE: PCT Int. Appl., 111 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000078800	A2	20001228	WO 2000-US16763	20000616
WO 2000078800	A3	20010719		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 1999-140258P P 19990618

AB Disclosed are surprisingly effective compns., therapeutic kits and vaccines comprising one or more Borrelia decorin binding protein components and one or more Borrelia outer surface protein components. Methods and medical uses are also disclosed in which the compns., kits and vaccines are administered to prevent and/or treat Borrelial infections, notably the Borrelial infections that cause **Lyme** disease.

L15 ANSWER 5 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:799217 HCAPLUS
DOCUMENT NUMBER: 134:53544
TITLE: Spirochete periplasmic flagella and motility
AUTHOR(S): Li, Chunhao; Motaleb, Md. Abdul; Sal, Melanie; Goldstein, Stuart F.; Charon, Nyles W.
CORPORATE SOURCE: Department of Microbiology and Immunology, Health Sciences Center, West Virginia University, Morgantown, WV, 26506-9177, USA
SOURCE: Journal of Molecular Microbiology and Biotechnology

(2000), 2(4), 345-354
CODEN: JMMBFF; ISSN: 1464-1801
PUBLISHER: Horizon Scientific Press
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with 94 refs. Spirochetes have a unique structure, and as a result their motility is different from that of other bacteria. They also have a special attribute: spirochetes can swim in a highly viscous, gel-like medium, such as that found in connective tissue, that inhibits the motility of most other bacteria. In spirochetes, the organelles for motility, the periplasmic flagella, reside inside the cell within the periplasmic space. A given periplasmic flagellum is attached only at one end of the cell, and depending on the species, may or may not overlap in the center of the cell with those attached at the other end. The no. of periplasmic flagella varies from species to species. These structures have been shown to be directly involved in spirochete motility, and they function by rotating within the periplasmic space. The mechanics of motility also vary among the spirochetes. In Leptospira, a motility model developed several years ago has been extensively tested, and the evidence supporting this model is convincing. Borrelia **burgdorferi** swims differently, and a model of its motility has been recently put forward. This model is based on analyzing the motion of swimming cells, high voltage electron microscopy of fixed cells, and mutant anal. To better understand spirochete motility on a more mol. level, the proteins and genes involved in motility are being analyzed. Spirochete periplasmic flagellar filaments are among the most complex of bacterial flagella. They are composed of the **FlaA** sheath proteins, and in many species, multiple FlaB core proteins. Allelic exchange mutagenesis of the genes which encode these proteins is beginning to yield important information with respect to periplasmic flagellar structure and function. Although we are at an early stage with respect to analyzing the function, organization, and regulation of many of the genes involved in spirochete motility, unique aspects have already become evident. Future studies on spirochete motility should be exciting, as only recently have complete genome sequences and tools for allelic exchange mutagenesis become available.

REFERENCE COUNT: 94 THERE ARE 94 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 6 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:539670 HCAPLUS
DOCUMENT NUMBER: 134:114451
TITLE: Quantitative approach for the serodiagnosis of canine **Lyme** disease by the immunoblot procedure
AUTHOR(S): Guerra, Marta A.; Walker, Edward D.; Kitron, Uriel
CORPORATE SOURCE: Department of Veterinary Pathobiology, University of Illinois College of Veterinary Medicine, Urbana, IL, 61802, USA
SOURCE: Journal of Clinical Microbiology (2000), 38(7), 2628-2632
CODEN: JCMIDW; ISSN: 0095-1137
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Serum samples obtained from healthy, asymptomatic dogs in areas of Wisconsin and northern Illinois where **Lyme** disease is endemic or non-endemic were assayed for antibodies to Borrelia **burgdorferi** by ELISA, and pos. results were confirmed by immunoblot assay. The authors found that 56.9% (562 of 1077) of the samples were pos. by ELISA and 82.0% (461 of 562) were pos. by immunoblotting. A logistic regression model was developed to distinguish between non-vaccinated dogs naturally infected with B. **burgdorferi** from areas where the disease is endemic and dogs from areas where the disease is non-endemic that were vaccinated against **Lyme** disease. Of the 18 protein bands analyzed, 8 were significantly different between the two groups. P93, p34, p31, and p28 occurred with increased frequency in vaccinated dogs, while p58, **p37**, p35, and p30 occurred more frequently in naturally infected dogs. The logistic regression equation obtained was used to det. the probability of natural infection among vaccinated dogs residing in areas where the disease is endemic. Of 125 samples, 87.2% had a very low probability of natural infection and only 2.4% were highly likely to be infected. Logistic regression is a useful method for distinguishing between vaccinated and naturally infected dogs and predicting the serol. status of vaccinated dogs from areas where **Lyme** disease is endemic.

REFERENCE COUNT: 33 THERE ARE 33 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 7 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:438465 HCAPLUS
DOCUMENT NUMBER: 133:174476
TITLE: **Lyme** arthritis resolution with antiserum to a 37-kilodalton Borrelia **burgdorferi** protein
AUTHOR(S): Feng, Sunlian; Hodzic, Emir; Barthold, Stephen W.
CORPORATE SOURCE: Center for Comparative Medicine, Schools of Medicine and Veterinary Medicine, University of California, Davis, CA, 95616, USA
SOURCE: Infect. Immun. (2000), 68(7), 4169-4173
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A 37-kDa protein from Borrelia **burgdorferi** (the agent of **Lyme** disease) was identified as a target for immune-mediated resoln. of **Lyme** arthritis. Studies in a mouse model have shown that arthritis resoln. can be mediated by antibodies (against unknown target antigens) within immune sera from actively infected mice. Immune sera from infected mice were therefore used to screen a B. **burgdorferi** genomic expression library. A gene was identified whose native product is a putative lipoprotein of approx. 37 kDa, referred to here as arthritis-related protein (Arp). Active and passive immunization of mice with recombinant Arp or Arp antiserum, resp., did not protect mice from challenge inoculation. However, when Arp antiserum was administered to severe combined immunodeficient (SCID) mice with established infections and with ongoing arthritis and carditis, treatment selectively induced arthritis resoln. without affecting the status of carditis or influencing the status of infection, including spirochetemia. The selective arthritis-resolving effect of Arp antiserum mimics the

activity of immune serum from immunocompetent mice when such serum is transferred into SCID mice with established infections. The arp gene could not be amplified from unrelated B. **burgdorferi** isolates but hybridized with those isolates only under very-low-stringency conditions. Arp antiserum reacted against proteins of similar size in a wide range of B. **burgdorferi** isolates.

IT **288166-96-1**
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; nucleotide and deduced amino acid sequences of a B. **burgdorferi** gene that encodes a 37-kDa lipoprotein and **Lyme** arthritis resoln. with an antiserum to this protein)

IT **223093-03-6**, GenBank AF035553
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(nucleotide sequence; nucleotide and deduced amino acid sequences of a B. **burgdorferi** gene that encodes a 37-kDa lipoprotein and **Lyme** arthritis resoln. with an antiserum to this protein)

REFERENCE COUNT: 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 8 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:353310 HCAPLUS
DOCUMENT NUMBER: 134:3778
TITLE: Serologic diagnosis of **Lyme** borreliosis by using enzyme-linked immunosorbent assays with recombinant antigens
AUTHOR(S): Magnarelli, Louis A.; Ijdo, Jacob W.; Padula, Steven J.; Flavell, Richard A.; Fikrig, Erol
CORPORATE SOURCE: Department of Entomology, The Connecticut Agricultural Experiment Station, New Haven, CT, 06504, USA
SOURCE: Journal of Clinical Microbiology (2000), 38(5), 1735-1739
CODEN: JCMIDW; ISSN: 0095-1137
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Class-specific enzyme-linked immunosorbent assays (ELISAs) with purified recombinant antigens of Borrelia **burgdorferi** sensu stricto and Western blot analyses with whole cells of this spirochete were used to test human sera to det. which antigens were diagnostically important. In analyses for IgM antibodies, 14 (82%) of 17 serum samples from persons who had erythema migrans reacted pos. by an ELISA with one or more recombinant antigens. There was frequent antibody reactivity to protein 41-G (p41-G), outer surface protein C (OspC), and OspF antigens. In an ELISA for IgG antibodies, 13 (87%) of 15 serum samples had antibodies to recombinant antigens; reactivity to p22, p39, p41-G, OspC, and OspF antigens was frequent. By both ELISAs, serum specimens pos. for OspB, OspE, and **p37** were uncommon. Analyses of sera obtained from persons who were suspected of having human granulocytic ehrlichiosis (HGE) but who lacked antibodies to ehrlichiae revealed IgM antibodies to all recombinant antigens of B. **burgdorferi** except OspB and IgG antibodies to all antigens except OspE. Immunoblotting of sera from the study group of individuals suspected of having HGE reaffirmed antibody reactivity to

multiple antigens of B. **burgdorferi**. There was minor cross-reactivity when sera from healthy subjects or persons who had syphilis, oral infections, or rheumatoid arthritis were tested by ELISAs with **p37**, p41-G, OspB, OspC, OspE, and OspF antigens. Although the results of class-specific ELISAs with recombinant antigens were comparable to those recorded for assays with whole-cell antigen and for individuals with confirmed clin. diagnoses of **Lyme** borreliosis, immunoblotting is still advised as an adjunct procedure, particularly when there are low antibody titers by an ELISA.

REFERENCE COUNT: 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT.

L15 ANSWER 9 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:451378 HCAPLUS
DOCUMENT NUMBER: 131:83993
TITLE: Identification of **P37** antigen as **FlaA**, and the use of recombinant **P37** as a diagnostic reagent for **Lyme** disease
INVENTOR(S): Gilmore, Robert D., Jr.; Johnson, Barbara J. B.
PATENT ASSIGNEE(S): Biomerieux, Inc., USA
SOURCE: PCT Int. Appl., 34 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9935272	A1	19990715	WO 1999-US196	19990106
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9922133	A1	19990726	AU 1999-22133	19990106
EP 963438	A1	19991215	EP 1999-902061	19990106
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
BR 9904800	A	20000516	BR 1999-4800	19990106
JP 2001516458	T2	20010925	JP 1999-536287	19990106
PRIORITY APPLN. INFO.:			US 1998-4395 A	19980108
			WO 1999-US196 W	19990106

AB The invention provides reagents and improved methods for serodiagnosis of **Lyme** disease, particularly in persons with recently acquired infection. The invention describes the definitive identification of the antigen **P37** as **FlaA**, an outer sheath protein of the periplasmic flagella of Borrelia **burgdorferi**. The invention also demonstrates that **P37** (**FlaA**) is a prominent antigen in the early humoral immune response to B. **burgdorferi** infection and is significantly suitable for use in improved serol. tests

for exposure to **Lyme** disease spirochetes. The invention provides an assay for detecting **Lyme** disease infection comprising obtaining a serum sample from a patient, contacting said sample with recombinant **P37** (**FlaA**), and detecting any antibody specifically bound to said protein. In a preferred embodiment, the recombinant **P37** (**FlaA**) antigen is produced as a fusion protein with the **T7 gene 10** product.

IT **186616-82-0P 229479-88-3P**, Antigen **P37** (human gene **flaA**)
RL: ARG (Analytical reagent use); BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPN (Biosynthetic preparation); PRP (Properties); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES (Uses)
(amino acid sequence; identification of **P37** antigen as **FlaA**, and the use of recombinant **P37** as a diagnostic reagent for **Lyme** disease)

IT **181695-11-4**
RL: ARU (Analytical role, unclassified); BOC (Biological occurrence); PRP (Properties); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)
(nucleotide sequence; identification of **P37** antigen as **FlaA**, and the use of recombinant **P37** as a diagnostic reagent for **Lyme** disease)

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 10 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:173125 HCAPLUS
DOCUMENT NUMBER: 131:2393
TITLE: The Borrelia **burgdorferi** 37-kilodalton immunoblot band (**P37**) used in serodiagnosis of early **Lyme** disease is the **flaA** gene product
AUTHOR(S): Gilmore, Robert D., Jr.; Murphree, Rendi L.; James, Angela M.; Sullivan, Sarah A.; Johnson, Barbara J. B.
CORPORATE SOURCE: Division of Vector-Borne Infectious Diseases, U.S. Department of Health and Human Services, Fort Collins, CO, USA
SOURCE: J. Clin. Microbiol. (1999), 37(3), 548-552
CODEN: JCMIDW; ISSN: 0095-1137
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The 37-kDa protein (**P37**) of Borrelia **burgdorferi** is an antigen that elicits an early IgM (IgM) antibody response in **Lyme** disease patients. The **P37** gene was cloned from a B. **burgdorferi** genomic library by screening with antibody from a **Lyme** disease patient who had developed a prominent humoral response to the **P37** antigen. DNA sequence anal. of this clone revealed the identity of **P37** to be **FlaA**, an outer sheath protein of the periplasmic flagella. Recombinant **P37** expression was accomplished in Escherichia coli by using a gene construct with the leader peptide deleted and fused to a 38-kDa E. coli protein. The recombinant antigen was reactive in IgM immunoblots using serum

samples from patients clin. diagnosed with early **Lyme** disease that had been scored pos. for B. **burgdorferi** anti-**P37** reactivity. **Lyme** disease patient samples serol. neg. for the B. **burgdorferi P37** protein did not react with the recombinant. Recombinant **P37** may be a useful component of a set of defined antigens for the serodiagnosis of early **Lyme** disease. This protein can be utilized as a marker in diagnostic immunoblots, aiding in the standardization of the present generation of IgM serol. tests.

REFERENCE COUNT: 24 THERE ARE 24 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 11 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:50456 HCAPLUS
DOCUMENT NUMBER: 130:94463
TITLE: Borrelia **burgdorferi** P35 and **P37** proteins, expressed in vivo, elicit protective immunity. [Erratum to document cited in CA127:107928]
AUTHOR(S): Fikrig, Erol; Barthold, Stephen W.; Sun, Wei; Feng, Wen; Telford, Sam R., III; Flavell, Richard A.
CORPORATE SOURCE: Section Rheumatology, Dep. Internal Med., Yale Univ. Sch. Med., New Haven, CT, 06520, USA
SOURCE: Immunity (1998), 9(5), No pp. Given
CODEN: IUNIEH; ISSN: 1074-7613
PUBLISHER: Cell Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The initial portion of the p35 sequence reported in this paper is not a Borrelia **burgdorferi** sequence. The first 114 nucleotides of the p35 sequence are part of the pBluescript vector sequence. The remaining 813 nucleotides of the gene are correct. The complete gene sequence (see Fraser C. M., et al. [1997]. Genomic sequence of a **Lyme** disease spirochaete, Borrelia **burgdorferi**. Nature 390, 580-586) is designated as B. **burgdorferi** B31 open reading frame BBK32.

IT **192274-47-8**
RL: BPR (Biological process); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(amino acid sequence; Borrelia **burgdorferi** p35 and **p37** gene sequences, recognition of P35 and **P37** proteins by infected mice, serum from human **Lyme** disease patients, and elicitation of protective immunity P35 and **P37** proteins (Erratum))

IT **192274-48-9**
RL: PRP (Properties)
(amino acid sequence; Borrelia **burgdorferi** p35 and **p37** gene sequences, recognition of P35 and **P37** proteins by infected mice, serum from human **Lyme** disease patients, and elicitation of protective immunity P35 and **P37** proteins (Erratum))

IT **192273-97-5**
RL: BPR (Biological process); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(nucleotide sequence; Borrelia **burgdorferi** p35 and **p37** gene sequences, recognition of P35 and **P37** proteins by infected mice, serum from human **Lyme** disease

patients, and elicitation of protective immunity P35 and **P37** proteins (Erratum))

IT **192273-98-6**
RL: PRP (Properties)
(nucleotide sequence; Borrelia **burgdorferi** p35 and **p37** gene sequences, recognition of P35 and **P37** proteins by infected mice, serum from human **Lyme** disease patients, and elicitation of protective immunity P35 and **P37** proteins (Erratum))

L15 ANSWER 12 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:25029 HCAPLUS
DOCUMENT NUMBER: 130:195645
TITLE: The immunoglobulin (IgG) antibody response to OspA and OspB correlates with severe and prolonged **Lyme** arthritis and the IgG response to P35 correlates with mild and brief arthritis
AUTHOR(S): Akin, Evren; McHugh, Gail L.; Flavell, Richard A.; Fikrig, Erol; Steere, Allen C.
CORPORATE SOURCE: Divisions of Rheumatology/Immunology and Pediatric Rheumatology, New England Medical Center, Tupper Research Institute, Tufts University School of Medicine, Boston, MA, 02111, USA
SOURCE: Infect. Immun. (1999), 67(1), 173-181
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB In an effort to implicate immune responses to specific Borrelia **burgdorferi** proteins that may have a role in chronic **Lyme** arthritis, the authors studied the natural history of the antibody response to B. **burgdorferi** in serial serum samples from 25 patients monitored throughout the course of **Lyme** disease. In these patients, the IgM and IgG antibody responses to 10 recombinant B. **burgdorferi** proteins, detd. during early infection, early arthritis, and maximal arthritis, were correlated with the severity and duration of maximal arthritis. The earliest responses were usually to outer surface protein C (OspC), P35, **P37**, and P41; reactivity with OspE, OspF, P39, and P93 often developed weeks later; and months to years later, 64% of patients had responses to OspA and OspB. During early infection and early arthritis, the levels of IgG antibody to P35 correlated inversely with the subsequent severity or duration of maximal arthritis. In contrast, during periods of maximal arthritis, the levels of IgG antibody to OspA and OspB, esp. to a C-terminal epitope of OspA, correlated directly with the severity and duration of arthritis. Thus, the higher the IgG antibody response to P35 earlier in the infection, the milder and briefer the subsequent arthritis, whereas during maximal arthritis, the higher the IgG response to OspA and OspB, the more severe and prolonged the arthritis.
REFERENCE COUNT: 45 THERE ARE 45 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 13 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:681778 HCAPLUS

DOCUMENT NUMBER: 130:79613
TITLE: Differential expression of Borrelia **burgdorferi** genes during erythema migrans and **Lyme** arthritis
AUTHOR(S): Fikrig, Erol; Feng, Wen; Aversa, John; Schoen, Robert T.; Flavell, Richard A.
CORPORATE SOURCE: Departments of Internal Medicine and Orthopedics and Sections of Rheumatology and Immunobiology, Yale University School of Medicine, New Haven, CT, 06520-8031, USA
SOURCE: J. Infect. Dis. (1998), 178(4), 1198-1201
CODEN: JIDIAQ; ISSN: 0022-1899
PUBLISHER: University of Chicago Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Borrelia **burgdorferi**, the agent of **Lyme** disease, selectively expresses genes in the arthropod vector and mammalian host. Specific B. **burgdorferi** gene expression during human infection was examd. in tissue specimens, using RNA-polymerase chain reaction, from 3 patients with **Lyme** disease. OspA was investigated because OspA is down-regulated by B. **burgdorferi** in ticks during engorgement and is a vaccine candidate in phase III clin. trials. P35 and **p37** were also assessed because these genes are induced by spirochetes during murine **Lyme** borreliosis and play roles in protective immunity. P35 and **p37** mRNA were detected in erythema migrans biopsy specimens from 2 patients and in the synovium of 1 patient with **Lyme** arthritis. OspA mRNA was not identified in any of these tissues. These data show that ospA is repressed while p35 and **p37** are induced in human infection; these results are the first direct demonstration of differential B. **burgdorferi** gene expression during **Lyme** disease.

REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L15 ANSWER 14 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:472384 HCAPLUS
DOCUMENT NUMBER: 129:104912
TITLE: Complete genome sequence of Treponema pallidum, the syphilis spirochete
AUTHOR(S): Fraser, Claire M.; Norris, Steven J.; Weinstock, George M.; White, Owen; Sutton, Granger G.; Dodson, Robert; Gwinn, Michelle; Hickey, Erin K.; Clayton, Rebecca; Ketchum, Karen A.; Sodergren, Erica; Hardham, John M.; McLeod, Michael P.; Salzberg, Steven; Peterson, Jeremy; Khalak, Hanif; Richardson, Delwood; Howell, Jerrilyn K.; Chidambaram, Monjula; Utterback, Teresa; McDonald, Lisa; Artiach, Patricia; Bowman, Cheryl; Cotton, Matthew D.; Fujii, Claire; Garland, Stacey; Hatch, Bonnie; Horst, Kurt; Roberts, Kevin; Sandusky, Mina; Weidman, Janice; Smith, Hamilton O.; Venter, J. Craig
CORPORATE SOURCE: The Inst. Genomic Res., Rockville, MD, 20850, USA
SOURCE: Science (Washington, D. C.) (1998), 281(5375), 375-388
CODEN: SCIEAS; ISSN: 0036-8075

PUBLISHER: American Association for the Advancement of Science
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The complete genome sequence of Treponema pallidum was detd. and shown to be 1,138,006 base pairs contg. 1041 predicted coding sequences (open reading frames). Systems for DNA replication, transcription, translation, and repair are intact, but catabolic and biosynthetic activities are minimized. The no. of identifiable transporters is small, and no phosphoenolpyruvate:phosphotransferase carbohydrate transporters were found. Potential virulence factors include a family of 12 potential membrane proteins and several putative hemolysins. Comparison of the T. pallidum genome sequence with that of another pathogenic spirochete, Borrelia **burgdorferi**, the agent of **Lyme** disease, identified unique and common genes and substantiates the considerable diversity obsd. among pathogenic spirochetes.
IT **131094-19-4**, Flagellin (Treponema pallidum clone pRI17 gene **flaA** subunit precursor) **209615-26-9**
RL: PRP (Properties)
(amino acid sequence; complete genome sequence of Treponema pallidum)

L15 ANSWER 15 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1998:297614 HCAPLUS
DOCUMENT NUMBER: 129:38535
TITLE: Structure and expression of the **FlaA** periplasmic flagellar protein of Borrelia **burgdorferi**
AUTHOR(S): Ge, Yigong; Li, Chunhao; Corum, Linda; Slaughter, Clive A.; Charon, Nyles W.
CORPORATE SOURCE: Department of Microbiology and Immunology, Robert C. Byrd Health Sciences Center, West Virginia University, Morgantown, WV, 26506-9177, USA
SOURCE: J. Bacteriol. (1998), 180(9), 2418-2425
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The spirochete which causes **Lyme** disease, Borrelia **burgdorferi**, has many features common to other spirochete species. Outermost is a membrane sheath, and within this sheath are the cell cylinder and periplasmic flagella (PFs). The PFs are subterminally attached to the cell cylinder and overlap in the center of the cell. Most descriptions of the B. **burgdorferi** flagellar filaments indicate that these organelles consist of only one flagellin protein (FlaB). In contrast, the PFs from other spirochete species are comprised of an outer layer of **FlaA** and a core of FlaB. The authors found that a **flaA** homolog was expressed in B. **burgdorferi** and that it mapped in a fla/che operon. These results led them to analyze the PFs and **FlaA** of B. **burgdorferi** in detail. Using Triton X-100 to remove the outer membrane and isolate the PFs, we found that the 38.0-kDa **FlaA** protein purified with the PFs in assocn. with the 41.0-kDa FlaB protein. On the other hand, purifying the PFs by using Sarkosyl resulted in no **FlaA** in the isolated PFs. Sarkosyl has been used by others to purify B. **burgdorferi** PFs, and our results explain in part their failure to find **FlaA**. Unlike other spirochetes,

B. **burgdorferi FlaA** was expressed at a lower level than FlaB. In characterizing **FlaA**, it was found that it was posttranslationally modified by glycosylation, and thus it resembles its counterpart from Serpulina hyodysenteriae. We also tested if **FlaA** was synthesized in a spontaneously occurring PF mutant of B. **burgdorferi** (HB19Fla-). Although this mutant still synthesized **flaA** message in amts. similar to the wild-type amts., it failed to synthesize **FlaA** protein. These results suggest that, in agreement with data found for FlaB and other spirochete flagellar proteins, **FlaA** is likely to be regulated on the translational level. Western blot anal. using Treponema pallidum anti-**FlaA** serum indicated that **FlaA** was antigenically well conserved in several spirochete species. These results indicate that both **FlaA** and FlaB comprise the PFs of B. **burgdorferi** and that they are regulated differently from flagellin proteins of other bacteria.

L15 ANSWER 16 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:804175 HCAPLUS
DOCUMENT NUMBER: 128:85027
TITLE: Genomic sequence of a **Lyme** disease spirochaete, Borrelia **burgdorferi**
AUTHOR(S): Fraser, Claire M.; Casjens, Sherwood; Huang, Wai Mun; Sutton, Granger G.; Clayton, Rebecca; Lathigra, Raju; White, Owen; Ketchum, Karen A.; Dodson, Robert; Hickey, Erin K.; Gwinn, Michelle; Dougherty, Brian; Tomb, Jean-Francois; Fleischmann, Robert D.; Richardson, Delwood; Peterson, Jeremy; Kerlavage, Anthony R.; Quackenbush, John; Salzberg, Steven; Hanson, Mark; van Vugt, Rene; Palmer, Nanette; Adams, Mark D.; Gocayne, Jeannine; Weidman, Janice; Utterback, Teresa; Watthey, Larry; McDonald, Lisa; Artiach, Patricia; Bowman, Cheryl; Garland, Stacey; Fujii, Claire; Cotton, Matthew D.; Horst, Kurt; Roberts, Kevin; Hatch, Bonnie; Smith, Hamilton O.; Venter, J. Craig
CORPORATE SOURCE: Inst. Genomic Research, Rockville, MD, 20850, USA
SOURCE: Nature (London) (1997), 390(6660), 580-586
CODEN: NATUAS; ISSN: 0028-0836
PUBLISHER: Macmillan Magazines
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The genome of the bacterium Borrelia **burgdorferi** B31, the etiol. agent of **Lyme** disease, contains a linear chromosome of 910,725 base pairs and at least 17 linear and circular plasmids with a combined size of more than 533,000 base pairs. The chromosome contains 853 genes encoding a basic set of proteins for DNA replication, transcription, translation, solute transport and energy metab., but, like Mycoplasma genitalium, it contains no genes for cellular biosynthetic reactions. Because B. **burgdorferi** and M. genitalium are distantly related eubacteria, we suggest that their limited metabolic capacities reflect convergent evolution by gene loss from more metabolically competent progenitors. Of 430 genes on 11 plasmids, most have no known biol. function; 39% of plasmid genes are paralogs that form 47 gene families. The biol. significance of the multiple plasmid-encoded genes is not clear,

although they may be involved in antigenic variation or immune evasion.

IT **132989-37-8**, Protein (Borrelia **burgdorferi** plasmid clone pTRH32 gene ospA) **147925-62-0**, DNA formation factor (Borrelia **burgdorferi** clone pKK11/pKK14/pKK16 gene grpE) **147925-63-1**, Protein (Borrelia **burgdorferi** clone pKK11/pKK14/pKK16 gene dnaK reduced) **148847-97-6**, Protein (Borrelia **burgdorferi** strain ATCC 35210 clone pUC-B31/ospCL+ gene ospC precursor reduced) **148939-61-1 148972-53-6**, Protein L 34 (Borrelia **burgdorferi** strain 21 ribosome) **149291-84-9 150791-28-9**, Protein (Borrelia **burgdorferi** strain 212 99-amino acid protein gene) **151186-30-0**, DNA formation factor (Borrelia **burgdorferi** clone pB22 gene dnaA reduced) **161347-27-9 167360-74-9 170214-03-6 174663-62-8 177699-93-3 183326-93-4 183326-94-5 183326-95-6 183326-96-7 183326-98-9 183326-99-0 183327-01-7 183327-03-9 183327-04-0 183510-26-1 183510-31-8 184656-09-5 187758-10-7 200558-26-5 200558-28-7 200558-30-1 200558-32-3 200558-34-5 200558-36-7 200558-38-9 200558-40-3 200558-41-4 200558-42-5 200558-43-6 200650-72-2 200650-73-3 200650-74-4 200650-75-5 200650-76-6 200650-77-7 200650-78-8 200650-79-9 200650-80-2 200650-81-3 200650-82-4 200650-83-5 200650-84-6 200650-85-7 200650-86-8 200650-87-9 200650-88-0 200650-89-1 200650-90-4 200650-91-5 200650-92-6 200650-93-7 200650-94-8 200650-95-9 200650-96-0 200650-97-1 200650-98-2 200650-99-3 200651-00-9 200651-01-0 200651-02-1 200651-03-2 200651-04-3 200651-05-4 200651-06-5 200651-07-6 200651-08-7 200651-09-8 200651-10-1 200651-11-2 200651-12-3 200651-13-4 200651-14-5 200651-15-6 200651-16-7 200651-17-8 200651-18-9 200651-19-0 200651-20-3 200651-21-4 200651-22-5 200651-23-6 200651-24-7 200651-25-8 200651-26-9 200651-27-0 200651-28-1 200651-29-2 200651-30-5 200651-31-6 200651-32-7 200651-33-8 200651-34-9 200651-35-0 200651-36-1 200651-37-2 200651-38-3 200651-39-4 200651-40-7 200651-41-8 200651-42-9 200651-43-0 200651-44-1 200651-45-2 200651-46-3 200651-47-4 200651-48-5 200651-49-6 200651-50-9 200651-51-0 200651-52-1 200651-53-2 200651-54-3 200651-55-4 200651-56-5 200651-57-6 200651-58-7 200651-59-8 200651-60-1 200651-61-2 200651-62-3 200651-63-4 200651-64-5 200651-65-6 200651-66-7 200651-67-8 200651-68-9**

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RL: PRP (Properties)
(amino acid sequence; genomic sequence of a **Lyme** disease spirochaete, Borrelia **burgdorferi**)

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RL: PRP (Properties)
(amino acid sequence; genomic sequence of a **Lyme** disease spirochaete, Borrelia **burgdorferi**)

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RL: PRP (Properties)
(amino acid sequence; genomic sequence of a **Lyme** disease spirochaete, Borrelia **burgdorferi**)

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RL: PRP (Properties)
(amino acid sequence; genomic sequence of a **Lyme** disease spirochaete, Borrelia **burgdorferi**)

IT 200661-39-8 200661-40-1 200661-41-2
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RL: PRP (Properties)
(amino acid sequence; genomic sequence of a **Lyme** disease spirochaete, Borrelia **burgdorferi**)

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RL: PRP (Properties)
(amino acid sequence; genomic sequence of a **Lyme** disease spirochaete, Borrelia **burgdorferi**)

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RL: PRP (Properties)
(nucleotide sequence; genomic sequence of a **Lyme** disease spirochaete, Borrelia **burgdorferi**)

L15 ANSWER 17 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:746160 HCAPLUS
DOCUMENT NUMBER: 128:31107
TITLE: Borrelia **burgdorferi** proteins expressed during infection and methods and compositions for prevention, treatment, and diagnosis of **Lyme** disease
INVENTOR(S): Fikrig, Erol; Suk, Kyoungho; Barthold, Stephen W.; Flavell, Richard A.
PATENT ASSIGNEE(S): Yale University, USA; Fikrig, Erol; Suk, Kyoungho;

Barthold, Stephen W.; Flavell, Richard A.
SOURCE: PCT Int. Appl., 115 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9742325	A1	19971113	WO 1996-US6610	19960508
W: CA, JP, US				
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
EP 915977	A1	19990519	EP 1996-915650	19960508
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2000510339	T2	20000815	JP 1997-539871	19960508

PRIORITY APPLN. INFO.: WO 1996-US6610 W 19960508

AB Methods and compns. for the prevention, treatment and diagnosis of **Lyme** disease are disclosed. Novel B. **burgdorferi** proteins which are produced during infection of a host but are not expressed by in vitro cultures of B. **burgdorferi**, as well as genes for these proteins, are identified. Vaccines comprising the novel B. **burgdorferi** proteins, antibodies to these proteins, and diagnostic use of the proteins and antibodies are claimed. A method for identifying bacterial genes that are selectively expressed in vivo is also disclosed. Genes expressed during infection of mice but not expressed by in vitro cultures of B. **burgdorferi** were identified by differential screening of a B. **burgdorferi** DNA library with antisera from mice inoculated with killed B. **burgdorferi** and with antisera from mice infected with live B. **burgdorferi** spirochetes. Several genes were isolated and sequenced. The genes for the P21, P35 and **P37** proteins were mapped to sep. plasmids. Antibodies to these proteins were prepd. A humoral response to P35 and **P37** was detected in humans infected with B. **burgdorferi**. S.c. injection of mice with P35 and **P37** may protect mice from challenge with B. **burgdorferi**, but P21 did not protect mice from tick-mediated infection.

IT **192274-47-8 192274-48-9 199746-41-3**
RL: PRP (Properties)
(amino acid sequence; Borrelia **burgdorferi** proteins expressed during infection and methods and compns. for prevention, treatment, and diagnosis of **Lyme** disease)

IT **199746-39-9**
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(amino acid sequence; Borrelia **burgdorferi** proteins expressed during infection and methods and compns. for prevention, treatment, and diagnosis of **Lyme** disease)

IT **192273-97-5 192273-98-6 199746-38-8 199746-40-2 199746-42-4**
RL: PRP (Properties)
(nucleotide sequence; Borrelia **burgdorferi** proteins expressed during infection and methods and compns. for prevention, treatment, and

diagnosis of **Lyme** disease)

L15 ANSWER 18 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:504645 HCAPLUS
DOCUMENT NUMBER: 127:215753
TITLE: Molecular characterization of a flagellar/chemotaxis operon in the spirochete Borrelia **burgdorferi**
AUTHOR(S): Ge, Yigong; Charon, Nyles W.
CORPORATE SOURCE: Department of Microbiology and Immunology, West Virginia University, Robert C. Byrd Health Sciences Center, P.O. Box 9177, Morgantown, WV, 26506-9177, USA
SOURCE: FEMS Microbiol. Lett. (1997), 153(2), 425-431
CODEN: FMLED7; ISSN: 0378-1097
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A chemotaxis gene cluster from Borrelia **burgdorferi**, the spirochete that causes **Lyme** disease, was cloned, sequenced, and analyzed. This cluster contained three chemotaxis gene homologs (cheA, cheW and cheY) and an open reading frame we identified as cheX. Although the major functional domains for B. **burgdorferi** CheW and CheY were well conserved, the size of cheW was significantly different from the homolog of other bacteria. Phylogenetic anal. of CheY indicated that B. **burgdorferi** constitutes a distinct branch with Treponema pallidum and is closely assocd. with Archea and Gram-pos. bacteria. RT-PCR anal. indicated that the chemotaxis genes and the upstream flagellar gene **flaA** constitute an operon. Western blot anal. using antibody to Escherichia coli CheA resulted in two reactive proteins in the cell lysates of B. **burgdorferi** that is consistent with two cheA homologs being present in this organism. The results taken together suggest both similarities and differences in the chemotaxis app. of B. **burgdorferi** compared to those of other bacteria.
IT **195010-16-3 195010-17-4 195010-18-5**
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(amino acid sequence; mol. characterization and sequence of a flagellar/chemotaxis operon in the spirochete Borrelia **burgdorferi**)
IT **178409-92-2**, GenBank U61498
RL: BOC (Biological occurrence); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(nucleotide sequence; mol. characterization and sequence of a flagellar/chemotaxis operon in the spirochete Borrelia **burgdorferi**)

L15 ANSWER 19 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:408637 HCAPLUS
DOCUMENT NUMBER: 127:148116
TITLE: **FlaA**, a putative flagellar outer sheath protein, is not an immunodominant antigen associated with **Lyme** disease
AUTHOR(S): Ge, Yigong; Charon, Nyles W.
CORPORATE SOURCE: Department of Microbiology and Immunology, West Virginia University, Morgantown, WV, 26506-9177, USA

SOURCE: Infect. Immun. (1997), 65(7), 2992-2995
CODEN: INFIBR; ISSN: 0019-9567
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **FlaA** was recently assocd. with flagellar filaments of Borrelia **burgdorferi**. The authors tested whether antibodies to this protein are a good indicator of infection, as antibodies to **FlaA** proteins in other spirochetal infections show an increase in titer. Although overprodn. of intact **FlaA** was highly toxic to Escherichia coli, truncated proteins which lacked the N-terminal signal sequence could be successfully overexpressed. Immunoblotting with sera from mammalian hosts infected with B. **burgdorferi** indicated that **FlaA** is not an immunodominant antigen in **Lyme** disease. However, sera from two patients reacted with both recombinant and native **FlaA** protein, suggesting that B. **burgdorferi** **FlaA** was antigenic and expressed in vivo.

L15 ANSWER 20 OF 22 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:355491 HCAPLUS
DOCUMENT NUMBER: 127:107928
TITLE: Borrelia **burgdorferi** P35 and **P37** proteins, expressed in vivo, elicit protective immunity
AUTHOR(S): Fikrig, Erol; Barthold, Stephen W.; Sun, Wei; Feng, Wen; Telford, Sam R., III; Flavell, Richard A.
CORPORATE SOURCE: Section Rheumatology, Dep. Internal Med., Yale Univ. Sch. Med., New Haven, CT, 06520, USA
SOURCE: Immunity (1997), 6(5), 531-539
CODEN: IUNIEH; ISSN: 1074-7613
PUBLISHER: Cell Press
DOCUMENT TYPE: Journal
LANGUAGE: English

AB P35 and **p37** are Borrelia **burgdorferi** genes encoding 35 and 37 kDa proteins. The gene products were identified by differential screening of a B. **burgdorferi** expression library with sera from B. **burgdorferi** infected- and B. **burgdorferi** -hyperimmunized mice. Northern blot and RT-PCR analyses confirmed that these genes were selectively expressed in vivo. ELISA, using P35 and **P37**, showed that infected mice (5 of 5, 100%) and patients (31 of 43, 72%) with **Lyme** borreliosis developed P35 or **P37** antibodies. Mice developed peak IgG titers to P35 and **P37** within 30 days, followed by decline. Mice given both P35 and **P37** antisera were protected from challenge with 102 B. **burgdorferi**, and P35 and **P37** antisera also afforded protection when administered 24 h after spirochete challenge. The use of in vivo-expressed antigens such as P35 and **P37** represents a new approach for **Lyme** disease serodiagnosis and for understanding the role of B. **burgdorferi**-specific immune responses in host immunity.

IT **192274-47-8**
RL: BPR (Biological process); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(amino acid sequence; Borrelia **burgdorferi** p35 and

p37 gene sequences, recognition of P35 and **P37** proteins by infected mice and serum from human **Lyme** disease patients, and elicitation of protective immunity by P35 and **P37** proteins)

IT **192274-48-9**
RL: PRP (Properties)
(amino acid sequence; Borrelia **burgdorferi** p35 and **p37** gene sequences, recognition of P35 and **P37** proteins by infected mice and serum from human **Lyme** disease patients, and elicitation of protective immunity by P35 and **P37** proteins)

IT **192273-97-5**
RL: BPR (Biological process); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(nucleotide sequence; Borrelia **burgdorferi** p35 and **p37** gene sequences, recognition of P35 and **P37** proteins by infected mice and serum from human **Lyme** disease patients, and elicitation of protective immunity by P35 and **P37** proteins)

IT **192273-98-6**
RL: PRP (Properties)
(nucleotide sequence; Borrelia **burgdorferi** p35 and **p37** gene sequences, recognition of P35 and **P37** proteins by infected mice and serum from human **Lyme** disease patients, and elicitation of protective immunity by P35 and **P37** proteins)

L15 ANSWER 21 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:298657 HCAPLUS
DOCUMENT NUMBER: 126:339490
TITLE: A cheA cheW operon in Borrelia **burgdorferi**, the agent of **Lyme** disease
AUTHOR(S): Trueba, G. A.; Old, I. G.; Saint Girons, I.; Johnson, R. C.
CORPORATE SOURCE: Department of Microbiology, University of Minnesota Medical School, Minneapolis, MN, 55455, USA
SOURCE: Res. Microbiol. (1997), 148(3), 191-200
CODEN: RMCREW; ISSN: 0923-2508
PUBLISHER: Elsevier
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Borrelia **burgdorferi** sensu stricto homologs of cheA and cheW were cloned and characterized. A combination of strategies such as polymerase chain reaction (PCR) using degenerate primers, random-primed gene walking PCR and construction of a .lambda. library were used to identify the putative cheA gene. Sequence anal. of the DNA fragments obtained from the CT strain identified a 2,592-bp open reading frame (ORF) encoding an 864-amino-acid protein with significant similarity (53-64.6% identical residues) to the CheA of several genera of eubacteria. In particular, hallmarks of a histidine kinase family were found such as the location of the histidine autophosphorylation domain very close to the N terminus and the nucleotide-binding site. A second ORF located immediately downstream from the putative borrelial cheA gene encoded a 195-amino-acid protein which displayed a high level of similarity to

bacterial CheW. Using reverse transcription PCR, we demonstrated that cheA and cheW form an operon with an upstream, unidentified ORF. The cheA and cheW homologs were located at 722-737 kbp, 738-768 kbp and 743-824 kpb on the linear chromosomes of B. **burgdorferi** sensu stricto, B. garinii and B. afzelii, resp. Identification of cheA and cheW is the first step toward elucidation of a possible role of chemotaxis in virulence of the **Lyme** disease borreliae.

IT **189960-04-1 189960-05-2**
RL: BAC (Biological activity or effector, except adverse); PRP (Properties); BIOL (Biological study)
(amino acid sequence; structural characterization of cheA cheW chemotaxis operon in Borrelia **burgdorferi**, the agent of **Lyme** disease)

IT **167324-36-9**, Genbank u28962 **169072-51-9**, Genbank u34384
RL: PRP (Properties)
(nucleotide sequence; structural characterization of cheA cheW chemotaxis operon in Borrelia **burgdorferi**, the agent of **Lyme** disease)

L15 ANSWER 22 OF 22 HCAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:53248 HCAPLUS
DOCUMENT NUMBER: 126:141814
TITLE: An unexpected **flaA** homolog is present and expressed in Borrelia **burgdorferi**
AUTHOR(S): Ge, Yigong; Charon, Nyles W.
CORPORATE SOURCE: Robert C. Byrd Health Sciences Center, West Virginia Univ., Morgantown, WV, 26506-9177, USA
SOURCE: J. Bacteriol. (1997), 179(2), 552-556
CODEN: JOBAAY; ISSN: 0021-9193
PUBLISHER: American Society for Microbiology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Most investigators have assumed that the periplasmic flagella (PFs) of B. **burgdorferi** are composed of only 1 flagellin protein. The PFs of most other spirochete spp. are complex: these PFs contain an outer sheath of **FlaA** proteins and a core filament of FlaB proteins. During an anal. of a chemotaxis gene cluster of B. **burgdorferi** 212, a **flaA** gene homolog with a deduced polypeptide having 54-58% similarity to **FlaA** from other spirochetes was found. Like other **FlaA** proteins, B. **burgdorferi FlaA** has a conserved signal sequence at its N terminus. Based on reverse transcription-PCR and primer extension anal., this **flaA** homolog and 5 chemotaxis genes constitute a motility-chemotaxis operon. Immunoblots using anti-**FlaA** serum from Treponema pallidum and a lysate of B. **burgdorferi** showed strong reactivity to a protein of 38.0 kDa, which is consistent with the expression of **flaA** in growing cells.

IT **186616-82-0**
RL: PRP (Properties)
(amino acid sequence; **flaA** homolog is present and expressed in Borrelia **burgdorferi**)

IT **181695-11-4**, GenBank u62900
RL: PRP (Properties)
(nucleotide sequence; **flaA** homolog is present and expressed

in Borrelia **burgdorferi**)

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               IDENT? OR SERODIAGN?)
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9/AB/1 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09965245 99014940 PMID: 9798206
Early detection of cutaneous lymphoma.
Abd-el-Baki J; Stefanato CM; Koh HK; Demierre MF; Foss FM
Department of Dermatology, Boston University School of Medicine, Massachusetts, USA.
Oncology (UNITED STATES) Oct 1998 , 12 (10) p1521-30; discussion 1532-4, ISSN 0890-9091 Journal Code: AVP
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed
Cutaneous lymphomas comprise a spectrum of diseases characterized by infiltration of the skin by malignant lymphocytes. The clinical manifestations of cutaneous lymphomas vary, and they can mimic benign dermatoses, as well as nodal or visceral malignancies with cutaneous spread. Cutaneous lymphomas are divided into T -cell lymphomas and B-cell lymphomas. Cutaneous T -cell lymphomas include mycosis fungoides, Sezary syndrome, lymphomatoid papulosis, CD30+ large cell lymphoma, and adult T -cell leukemia/lymphoma. The extent and severity of skin manifestations in cutaneous T -cell lymphomas are prognostic indicators of extracutaneous involvement. Primary cutaneous B-cell lymphomas comprise 10 % to 25% of all primary cutaneous non-Hodgkin's lymphomas and are classified according to their cell of origin. Most cutaneous B-cell lymphomas have an indolent course and excellent prognosis when compared to their nodal counterparts. Many factors have been implicated in the etiology of cutaneous lymphomas, including chemical and drug exposures, as well as microbial agents, such as the Epstein-Barr virus (EBV), human T -lymphocyte virus-1 (HTLV-1), and Borrelia burgdorferi . Immunohistochemistry and lymphocyte-receptor gene rearrangement studies are useful in distinguishing malignant from benign conditions.

9/AB/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09918101 99023007 PMID: 9806060
Differential expression of Borrelia burgdorferi genes during erythema migrans and Lyme arthritis.
Fikrig E; Feng W; Aversa J; Schoen RT; Flavell RA
Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06520-8031, USA.
Journal of infectious diseases (UNITED STATES) Oct 1998 , 178 (4) p1198-201, ISSN 0022-1899 Journal Code: IH3
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Borrelia burgdorferi , the agent of Lyme disease, selectively expresses genes in the arthropod vector and mammalian host. Specific B. burgdorferi gene expression during human infection was examined in tissue specimens, using RNA-polymerase chain reaction, from 3 patients with Lyme disease. ospA was investigated because OspA is down-regulated by B. burgdorferi in ticks during engorgement and is a vaccine candidate in phase III clinical trials. p35 and p37 were also assessed because these genes are induced by spirochetes during murine Lyme borreliosis and play roles in protective immunity. p35 and p37 mRNA were detected in erythema migrans biopsy specimens from 2 patients and in the synovium of 1 patient with Lyme arthritis. ospA mRNA was not identified in any of these tissues. These data show that ospA is repressed while p35 and p37 are induced in human infection; these results are the first direct demonstration of differential

B. burgdorferi gene expression during Lyme disease.

9/AB/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09639281 98101460 PMID: 9440510
Chemotaxis in Borrelia burgdorferi.
Shi W; Yang Z; Geng Y; Wolinsky LE; Lovett MA
School of Dentistry, and Molecular Biology Institute, University of California, Los Angeles 90095-1668, USA. wenyuan@ucla.edu
Journal of bacteriology (UNITED STATES) Jan 1998 , 180 (2) p231-5, ISSN 0021-9193 Journal Code: HH3
Contract/Grant No.: 5-T32-AI-07323, AI, NIAID; AI-29733, AI, NIAID; GM54666, GM, NIGMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Borrelia burgdorferi is a motile spirochete which has been identified as the causative microorganism in Lyme disease. The physiological functions which govern the motility of this organism have not been elucidated. In this study, we found that motility of B. burgdorferi required an environment similar to interstitial fluid (e.g., pH 7.6 and 0.15 M NaCl). Several methods were used to detect and measure chemotaxis of B. burgdorferi . A number of chemical compounds and mixtures were surveyed for the ability to induce positive and negative chemotaxis of B. burgdorferi . Rabbit serum was found to be an attractant for B. burgdorferi , while ethanol and butanol were found to be repellents. Unlike some free-living spirochetes (e.g., Spirochaeta aurantia), B. burgdorferi did not exhibit any observable chemotaxis to common sugars or amino acids. A method was developed to produce spirochete cells with a self-entangled end. These cells enabled us to study the rotation of a single flagellar bundle in response to chemoattractants or repellents. The study shows that the frequency and duration for pausing of flagella are important for chemotaxis of B. burgdorferi .

9/AB/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09560142 97417814 PMID: 9271872
Molecular characterization of a flagellar/chemotaxis operon in the spirochete Borrelia burgdorferi.
Ge Y; Charon NW
Department of Microbiology and Immunology, West Virginia University, Robert C. Byrd Health Sciences Center, Morgantown 26506-9177, USA.
FEMS microbiology letters (NETHERLANDS) Aug 15 1997 , 153 (2) p425-31, ISSN 0378-1097 Journal Code: FML
Contract/Grant No.: AI29743, AI, NIAID
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
A chemotaxis gene cluster from Borrelia burgdorferi , the spirochete that causes Lyme disease, was cloned, sequenced, and analyzed. This cluster contained three chemotaxis gene homologs (cheA, cheW and cheY) and an open reading frame we identified as cheX. Although the major functional domains for B. burgdorferi CheW and CheY were well conserved, the size of cheW was significantly different from the homolog of other bacteria. Phylogenetic analysis of CheY indicated that B. burgdorferi constitutes a distinct branch with Treponema pallidum and is closely associated with Archea and Gram-positive bacteria. RT-PCR analysis indicated that the

chemotaxis genes and the upstream flagellar gene flaA constitute an operon. Western blot analysis using antibody to Escherichia coli CheA resulted in two reactive proteins in the cell lysates of B. burgdorferi that is consistent with two cheA homologs being present in this organism. The results taken together suggest both similarities and differences in the chemotaxis apparatus of B. burgdorferi compared to those of other bacteria.

9/AB/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09362711 97309538 PMID: 9166953
Microbiology of Borrelia burgdorferi.
Rosa PA
Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories of the NIAID, NIH, Hamilton, Montana 59840, USA.
Seminars in neurology (UNITED STATES) Mar 1997 , 17 (1) p5-10, ISSN 0271-8235 Journal Code: SEJ
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed
This article reviews the natural history, taxonomy, physical structure, growth requirements, and molecular structure of Borrelia burgdorferi sensu lato, the causative agent of Lyme disease. These spirochetal bacteria are maintained in nature through an infectious cycle between wild mammals and ticks. Borreliae are fastidious, slow-growing bacteria, found only in association with their arthropod or mammalian hosts in nature, and propagatable in the laboratory in a rich growth medium. The characteristic shape of borreliae is imposed by periplasmic flagella , located beneath the outer membrane and attached to the protoplasmic cylinder. The outer membrane of borreliae contains a number of abundant lipoproteins that are of serodiagnostic utility and currently under consideration as vaccine targets. The borrelial genome is unique in structure, organization, and copy number. Recent experiments demonstrate the feasibility of specific gene inactivation as a means with which to study the biology of borreliae and the pathogenesis of Lyme disease.

9/AB/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09331750 97318894 PMID: 9175831
Borrelia burgdorferi P35 and P37 proteins, expressed in vivo, elicit protective immunity.
Fikrig E; Barthold SW; Sun W; Feng W; Telford SR; Flavell RA
Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06520, USA.
Immunity (UNITED STATES) May 1997 , 6 (5) p531-9, ISSN 1074-7613 Journal Code: CCF
Contract/Grant No.: AI-26815, AI, NIAID; AI-30548, AI, NIAID; AR-40452, AR, NIAMS; +
Erratum in Immunity 1998 Nov;9(5) following 755
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
p35 and p37 are Borrelia burgdorferi genes encoding 35 and 37 kDa proteins. The gene products were identified by differential screening of a B. burgdorferi expression library with sera from B. burgdorferi infected- and B. burgdorferi -hyperimmunized mice. Northern blot and RT-PCR analyses confirmed that these genes were selectively expressed in

vivo. ELISA, using P35 and P37 , showed that infected mice (5 of 5, 100%) and patients (31 of 43, 72%) with Lyme borreliosis developed P35 or P37 antibodies. Mice developed peak IgG titers to P35 and P37 within 30 days, followed by decline. Mice given both P35 and P37 antisera were protected from challenge with 10(2) B. burgdorferi , and P35 and P37 antisera also afforded protection when administered 24 hr after spirochete challenge. The use of in vivo-expressed antigens such as P35 and P37 represents a new approach for Lyme disease serodiagnosis and for understanding the role of B. burgdorferi -specific immune responses in host immunity.

9/AB/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09162423 97160300 PMID: 9007816
Analysis of the human antibody response to outer surface protein C (OspC) of Borrelia burgdorferi sensu stricto, B. garinii, and B. afzelii.
Mathiesen MJ; Hansen K; Axelsen N; Halkier-Sorensen L; Theisen M
Borrelia Laboratory, Department of Clinical Biochemistry, Statens Seruminstitut, Copenhagen S, Denmark. mmathiesen@cb.diag.ssi.dk
Medical microbiology and immunology (GERMANY) Nov 1996 , 185 (3) p121-9, ISSN 0300-8584 Journal Code: M58
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The aim of this study was to determine by Western blotting (WB) the prevalence of anti-outer surface protein C (OspC) IgM and IgG antibodies in patients with Lyme borreliosis according to each of the three genospecies of Borrelia burgdorferi sensu lato. Strains of B. burgdorferi sensu stricto (MUL), B. garinii (DK 6), and B. afzelii (DK 26) served as antigen, all of which expressed abundant OspC. We examined sera from 117 patients with untreated early and late Lyme borreliosis, as well as from 100 blood donors and 29 patients with syphilis. WB results were compared with the B. burgdorferi flagellum enzyme-linked immunosorbent assay (ELISA) data. OspC from B. burgdorferi sensu stricto showed the lowest diagnostic sensitivity. OspC from B. garinii and B. afzelii performed almost identically in erythema migrans, with an IgM positive rate of 36% versus 34%, whereas OspC from B. garinii performed best in neuroborreliosis (60% versus 44%). The anti-OspC IgG response was less prominent than the IgM response and was infrequent in the late stages of the disease (0-20%). The benefit of combining the evaluation of anti-OspC responses with all three species was limited. The overall diagnostic sensitivity of WB anti-B. garinii OspC evaluation was, in the early stages of the disease, comparable to the results obtained using the flagellum ELISA. In erythema migrans and neuroborreliosis, the addition of anti-OspC IgM to the flagellum ELISA increased the sensitivity by 15% and 10%, respectively. It can, therefore, be concluded that OspC from B. garinii is a suitable OspC test antigen, and that supplementary use of OspC from other species adds little to the overall diagnostic sensitivity. An ELISA based on B. garinii OspC and native flagella seems currently the most promising concept for a future antibody test in early Lyme borreliosis.

9/AB/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09076708 97086621 PMID: 8932310
Structural analysis of the Leptospiraceae and Borrelia burgdorferi by high-voltage electron microscopy.
Goldstein SF; Buttle KF; Charon NW

Department of Genetics and Cell Biology, University of Minnesota, St. Paul 55108, USA.
Journal of bacteriology (UNITED STATES) Nov 1996 , 178 (22) p6539-45, ISSN 0021-9193 Journal Code: HH3
Contract/Grant No.: AI29743, AI, NIAID; DE04645, DE, NIDCR; DE12046, DE, NIDCR; +
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Spirochetes are an evolutionary and structurally unique group of bacteria. Outermost is a membrane sheath (OS), and within this sheath are the protoplasmic cell cylinder (PC) and periplasmic flagella (PFs). The PFs are attached at each end of the PC and, depending on the species, may or may not overlap in the center of the cell. The precise location of the PFs within the spirochetal cells is unknown. The PFs could lie along the cell axis. Alternatively, the PFs could wrap around the PC in either a right- or a left-handed sense. To understand the factors that cause the PFs to influence cell shape and allow the cells to swim, we determined the precise location of the PFs in the Leptospiraceae (Leptonema illini) and Borrelia burgdorferi . Our approach was to use high-voltage electron microscopy and analyze the three-dimensional images obtained from thick sections of embedded cells. We found that a single PF in L. illini is located in a central channel 29 nm in diameter running along the helix axis of the right-handed PC. The presence of the PFs is associated with the end being hook shaped. The results obtained agree with the current model of Leptospiraceae motility. In B. burgdorferi , which forms a flattened wave, the relationship between the PFs and the PC is more complicated. A multistrand ridge 67 nm in diameter, which was shown to be composed of PFs by cross-sectional and mutant analysis, was found to extend along the entire length of the cell. We found that the PFs wrapped around the PC in a right-handed sense. However, the PFs formed a left-handed helix in space. The wavelength of the cell body and the helix pitch of the PFs were found to be identical (2.83 microm). The results obtained were used to propose a model of B. burgdorferi motility whereby backward-propagating waves, which gyrate counterclockwise as viewed from the back of the cell, are generated by the counterclockwise rotation of the internal PFs. Concomitant with this motion, the cell is believed to rotate clockwise about the body axis as shown for the Leptospiraceae.

9/AB/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08357879 95190294 PMID: 7884218
Treatment of late Lyme borreliosis.
Wahlberg P; Granlund H; Nyman D; Panelius J; Seppala I
Department of Medicine, Aland Central Hospital, Mariehamn, Finland.
Journal of infection (ENGLAND) Nov 1994 , 29 (3) p255-61, ISSN 0163-4453 Journal Code: IG9
Languages: ENGLISH
Document type: Clinical Trial; Journal Article; Randomized Controlled Trial
Record type: Completed
The aim of this study was to develop a treatment for late Lyme borreliosis and to compare the clinical results with serological findings before and after treatment. It was done in the Aland Islands (population 25,000), a region endemic for Lyme borreliosis. The patients were the first consecutive 100 patients from the Aland Islands with late Lyme borreliosis. They were followed for at least 1 year after treatment. The clinical results of treatment were compared with results of analyses of flagellar IgG antibodies to Borrelia burgdorferi done at the time of diagnosis

before treatment and up to 12 months afterwards. Short periods of treatment were not generally effective. The outcome was successful in four of 13 treatments with 14 days of intravenous ceftriaxone alone, in 50 of 56 assessable treatments with ceftriaxone followed by 100 days of amoxycillin plus probenecid, and in 19 of 23 completed treatments with ceftriaxone followed by 100 days of cephadroxil. Titres of IgG antibodies to B. burgdorferi flagella declined significantly after 6 and 12 months in the patients who had successful treatments. All patients whose final titres were less than 30% of the initial titre were in the successful group. Their titres usually remained above the upper limit of normal for a long time but a decline to a value of less than 30% of that before treatment was always a sign of cure.

9/AB/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08357762 95189928 PMID: 7883886
Detection of Borrelia burgdorferi sensu lato in lesional skin of patients with erythema migrans and acrodermatitis chronica atrophicans by ospA-specific PCR.
Moter SE; Hofmann H; Wallich R; Simon MM; Kramer MD
Institute of Immunology, University of Heidelberg, Germany.
Journal of clinical microbiology (UNITED STATES) Dec 1994 , 32 (12) p2980-8, ISSN 0095-1137 Journal Code: HSH
Languages: ENGLISH
Document type: Clinical Trial; Journal Article
Record type: Completed
The aim of this study was to develop a sensitive and specific PCR for the detection of Borrelia burgdorferi DNA. The plasmid-located gene coding for the outer surface protein A (OspA [31-kDa protein]) was used as a target. Nucleotide sequence information from different B. burgdorferi ospA genotypes was used to design primers homologous to different genotypes. The sensitivity of the nested PCR differed from 1 fg to 1 pg of borrelial DNA, depending on the strain analyzed. No cross-reactions with DNA from spirochetes other than B. burgdorferi or with human DNA were observed. A total of 22 skin biopsy samples from patients with erythema migrans (EM [n = 10]) or acrodermatitis chronica atrophicans (ACA [n = 12]) were examined for the presence of B. burgdorferi by nested PCR. Of 22 biopsies, 80% from EM patients and 92% from ACA patients were positive by PCR amplification. By comparison, 50% of the EM patients had elevated B. burgdorferi -specific immunoglobulin M (IgM) and/or IgG antibody levels as tested by enzyme-linked immunosorbent assay (ELISA) using purified B. burgdorferi flagella as antigen. A total of 33% of ACA patients had elevated IgM titers, and all had high IgG titers in their sera. Only 30% of specimens from patients with EM and none from patients with ACA were positive by culture. All culture-positive specimens were also positive by PCR. Thus, the sensitivities of the PCR were 80 and 92%, respectively, for patients with EM and ACA on the basis of the clinical and histopathological diagnoses of Lyme disease.(ABSTRACT TRUNCATED AT 250 WORDS)

9/AB/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08161726 94219201 PMID: 8165888
Lyme neuroborreliosis: improvements of the laboratory diagnosis and a survey of epidemiological and clinical features in Denmark 1985-1990.
Hansen K
Department of Infection-Immunology, Statens Seruminstitut, Copenhagen, Denmark.

Acta neurologica Scandinavica. Supplementum (DENMARK) 1994 , 151 p1-44, ISSN 0065-1427 Journal Code: 1BU
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

Lyme neuroborreliosis (LNB) has within the last few years become one of the most frequent neuroinfections. This thesis is based on 7 publications which have two main topics: (i) to improve and develop laboratory methods for routine diagnosis of LNB, (ii) to generate epidemiological data for LNB and to achieve a descriptive clinical delimitation of this disease. Laboratory diagnosis in Lyme borreliosis is based on detection of a Borrelia burgdorferi (Bb) specific immune response. Up till now serological assays have not achieved a sufficient diagnostic specificity and sensitivity. This is partly due to the use of test antigens consisting of all proteins of the spirochete including broadly cross-reacting antigens. In order to improve diagnostic antibody detection we isolated an immunodominant structural protein of Bb, the motility organelle the flagellum. The use of native, morphologically intact flagella as test antigen in ELISA led to a significantly increased diagnostic specificity, and, especially in early disease, to an improved diagnostic sensitivity. Reservations regarding the use of only one out of the over 100 proteins of Bb as a diagnostic antigen probe are groundless. The early as well as the late immune response to Bb always includes antibodies to the flagellum. The Bb flagellum is as a test antigen not completely Bb specific. Compared with all other antigen preparations however, the flagellum is at present the best compromise, if a sensitive and specific routine serology is requested. The diagnostic performance of specific IgM detection was improved with a mu-capture ELISA, which used biotin labelled Bb flagella . Compared to conventional indirect ELISA this technique avoids false positive results due to IgM rheumatoid factor interference and false low or false negative results due to IgG competition for the test antigen. The antibody response in Bb infection develops slowly. Patients with LNB can be antibody negative in blood up to 6-8 weeks after onset of neurological symptoms. Longstanding but seronegative disease in untreated patients is unlikely to occur. Expectations of further improvement of Lyme borreliosis serology focuses presently on the performance of the outer surface protein (Osp) C as a test antigen and on the genus specific domain of the Bb flagellin. Theoretically this region constitutes the best candidate for a better test antigen either as a recombinant or a synthetic peptide. In LNB a prominent Bb specific intrathecal antibody response develops.(ABSTRACT TRUNCATED AT 400 WORDS)

9/AB/12 (Item 12 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08160182 94202188 PMID: 8151682
Diagnosis of Lyme borreliosis: non-specific serological reactions with Borrelia burgdorferi sonicate antigen caused by IgG2 antibodies.

Seppala IJ; Kroneld R; Schauman K; Forsen KO; Lassenius R
Department of Bacteriology and Immunology, University of Helsinki, Finland.

Journal of medical microbiology (SCOTLAND) Apr 1994 , 40 (4) p293-302, ISSN 0022-2615 Journal Code: J2N
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

ELISA methods that measure IgG class antibodies to sonicated Borrelia burgdorferi may give false positive results. These errors could be traced to non-specific reactivity in subclass IgG2 in several instances. Sera were sampled randomly from two adult populations, which differed in having a high and low incidence of Lyme disease. If the binding of IgG2 subclass

antibodies was left unrecorded in the test by the use of monoclonal reagent antibodies selective for IgG1 and IgG3, the frequency of positivity in the ELISA test decreased in samples from the low risk group. Twenty-one samples were found to be positive in an immunoblot confirmatory test. Correct prediction of positivity was obtained for 15 sera by ELISA restricted to IgG1 plus IgG3, for only four sera by ELISA restricted to IgG2 and for only six sera by IgG subclass non-restricted ELISA. A non-restricted ELISA with purified flagella of B. burgdorferi as the antigen predicted correctly 14 of the immunoblot-positive sera. The results of this ELISA correlated well with those of the IgG1 plus IgG3 subclass restricted ELISA in the high risk population (r = 0.95, prevalence of seropositivity 12%), but was significantly worse for the low risk group (r = 0.47, prevalence 2.9%). IgG subclass restriction also decreased cross-reactions of syphilitic sera in the ELISA with sonicated antigen.

9/AB/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08155456 94220804 PMID: 8167426
Characterization of antisera raised against Treponema denticola (ATCC 33521) whole cell, outer sheath, protoplasmic cylinder, and axial flagella.
Wolf V; Lange R
Robert Koch-Institut des BGA, Berlin, Germany.
Zentralblatt fur Bakteriologie (GERMANY) Jan 1994 , 280 (3) p325-31 , ISSN 0934-8840 Journal Code: BD7
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
In this study we produced polyclonal antisera directed to whole cell, outer sheath, protoplasmic cylinder and axial flagella sonicates of Treponema denticola (ATCC 33521) reference strain. Furthermore, the reactivity of the antisera was determined , using the enzyme-linked immunosorbent assay and immunoblotting techniques. As control antigen, other gram-negative bacteria (Salmonella minnesota, Escherichia coli) and related pathogenic spirochetes (Borrelia burgdorferi and Treponema pallidum) were used. It could be shown that the purified antibodies were specific for Treponema denticola and did not cross-react with the control antigens tested. Interestingly, with one exception, the anti-axial flagella antibody reacted with the flagellin of Treponema pallidum but not with Borrelia burgdorferi flagella . It is intended to use these antisera for the characterization of patient isolates in further studies.

9/AB/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07749068 92325211 PMID: 1378061
Mapping the major antigenic domains of the native flagellar antigen of Borrelia burgdorferi.
Jiang W; Luft BJ; Schubach W; Dattwyler RJ; Gorevic PD
Department of Medicine, State University of New York, Stony Brook 11794-8161.
Journal of clinical microbiology (UNITED STATES) Jun 1992 , 30 (6) p1535-40, ISSN 0095-1137 Journal Code: HSH
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Purified flagellar protein (p41) of Borrelia burgdorferi (strain B31) was subjected to chemical cleavage with hydroxylamine or proteolysis with V8 protease, endoproteinase Asp-N, or alpha-chymotrypsin. The resulting

polypeptides were identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and their positions in the published DNA sequence of the p41 protein were determined by amino-terminal sequencing and amino acid analysis. Epitope specificities of antibody binding by a monoclonal antibody raised by immunization of mice with purified flagella and pooled sera from patients with multiple erythema migrans, late Lyme borreliosis, or secondary syphilis were analyzed by Western blots (immunoblots) of peptides transferred to Immobilon polyvinylidene difluoride filters. The major epitope binding one murine monoclonal antibody (158) was localized to a carboxy-terminal domain that includes residues 300 to 336. The dominant epitopes binding human polyclonal antibodies are in the central portion of the molecule (residues 182 to 218) that is not conserved compared with other bacterial flagellins. Additional reactive epitopes were identified in the amino-terminal domain of the protein. Sera from patients with syphilis bound strongly to the amino-terminal conserved domain, providing a structural basis for cross-reactivity seen in standard enzyme-linked immunosorbent assays, but not to the central part of the molecule. Specific and cross-reactive antigenic determinants need to be considered in the design of improved immunodiagnostics for spirochetal diseases.

9/AB/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07676512 93117534 PMID: 1475520
Spirochete chemotaxis, motility, and the structure of the spirochetal periplasmic flagella.
Charon NW; Greenberg EP; Koopman MB; Limberger RJ
West Virginia University, Department of Microbiology and Immunology, Morgantown 26506.
Research in microbiology (FRANCE) Jul-Aug 1992 , 143 (6) p597-603, ISSN 0923-2508 Journal Code: R6F
Languages: ENGLISH
Document type: Journal Article; Review; Review, Tutorial
Record type: Completed
Spirochetes have a unique motility system that is characterized by flagellar filaments contained within the outer membrane sheath. Direct evidence using video microscopy has recently been obtained which indicates that these periplasmic flagella (PF) rotate in several spirochetal species. This rotation generates thrust. As shown for one spirochete, Spirochaeta aurantia, motility is driven by a proton motive force. Spirochete chemotaxis has been most thoroughly studied in S. aurantia. This spirochete exhibits three distinct behaviours, runs of smooth swimming, reversals and flexing. These behaviours are modulated by addition of attractants such that S. aurantia swims towards higher concentrations of attractants in a spatial gradient. Unlike the prototypical bacterium, Escherichia coli, chemotaxis in S. aurantia involves fluctuations in membrane potential. The PF of a number of spirochetes have been examined in considerable detail. For most species, the PF filaments are complex, consisting of an assembly of several different polypeptides. There are several antigenically related core polypeptides surrounded by an outer layer consisting of a different polypeptide. Borrelia burgdorferi and Spirochaeta zuelzerae represent exceptions where the filaments are composed of a single major polypeptide species. The genes encoding the filament polypeptides from several spirochete species have been cloned and analysed. Apparently, the outer layer polypeptides of S. aurantia, Treponema pallidum and Serpulina hyodysenteriae are transcribed from sigma-70-like promoters, whereas the core polypeptide genes are transcribed from sigma-28-like promoters. A gene encoding the hook polypeptide in Treponema phagedenis has been cloned and analysed. The product of this gene shows significant similarity to the E. coli hook protein, FlgE, and homologs have been

identified in T. pallidum and B. burgdorferi .(ABSTRACT TRUNCATED AT 250 WORDS).

9/AB/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07562767 92121121 PMID: 1732217
Morphology and dynamics of protruding spirochete periplasmic flagella.
Charon NW; Goldstein SF; Block SM; Curci K; Ruby JD; Kreiling JA; Limberger RJ
Department of Microbiology and Immunology, West Virginia University, Morgantown 26506.
Journal of bacteriology (UNITED STATES) Feb 1992 , 174 (3) p832-40, ISSN 0021-9193 Journal Code: HH3
Contract/Grant No.: AI29743, AI, NIAID; DE04645, DE, NIDCR; S07-RR05433-29, RR, NCRR; +
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
We recently characterized the three-dimensional shape of Treponema phagedenis periplasmic flagella (PFs). In the course of these studies, we observed protrusions on swimming cells that resembled PFs. Here we present a detailed characterization of the shape, structure, and motion of these protrusions. Although protrusion formation occurred primarily in wild-type cells during the stationary phase, a large fraction of exponential-phase cells of cell cylinder helicity mutants (greater than 90% of mutant T-52) had protrusions. These results suggest that cells bearing protrusions can still participate in cell division. T. phagedenis protrusions had the identical helix handedness, pitch, and diameter to those of purified PFs. Protrusions were not present on mutants unable to synthesize PFs, but were present in all motile revertants which regained PFs. These results, taken together with electron microscope observations, suggest that protrusions consist of PFs surrounded by an outer membrane sheath. To analyze protrusion movements, we held cells against a coverglass surface with optical tweezers and observed the motion of protrusions by video-enhanced differential interference contrast light microscopy. Protrusions were found to gyrate in both clockwise and counterclockwise directions, and direct evidence was obtained that protrusions rotate. Protrusions were also observed on Treponema denticola and Borrelia burgdorferi . These were also left-handed and had the same helix handedness, pitch, and diameter as purified PFs from their respective species. The PFs from T. denticola had a helix diameter of 0.26 microns and a helix pitch of 0.78 micron; PFs from B. burgdorferi had a helix diameter of 0.28 micron and a helix pitch of 1.48 microns.(ABSTRACT TRUNCATED AT 250 WORDS)

9/AB/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07453923 91378210 PMID: 1897911
Lyme neuroborreliosis: a new sensitive diagnostic assay for intrathecal synthesis of Borrelia burgdorferi--specific immunoglobulin G, A, and M.
Hansen K; Lebech AM
Borrelia Laboratory, Department of Infection-Immunology, Statens Seruminstitut, Copenhagen, Denmark.
Annals of neurology (UNITED STATES) Aug 1991 , 30 (2) p197-205, ISSN 0364-5134 Journal Code: 6AE
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

An antibody capture enzyme-linked immunosorbent assay was developed to measure directly intrathecal immunoglobulin (Ig) G, A, and M synthesis specific for Borrelia burgdorferi . Purified, biotin-avidin-peroxidase-lab eled B. burgdorferi flagella was used as test antigen. Paired cerebrospinal fluid and serum specimens from 100 patients with clinically definite neuroborreliosis and 35 control subjects with neurological diseases were examined. Significant B. burgdorferi -specific intrathecal IgG, A, and M production was found in 89%, 65% and 67% of patients with neuroborreliosis. Local synthesis of specific IgA was only seen in patients with significant local IgG synthesis. Antibody production in cerebrospinal fluid began by 2 weeks after onset of neurological symptoms. At the end of the second week specific IgM, IgG, or both, was detected in 88% of the patients. Specific IgG synthesis was present in all patients by 6 weeks after onset. Specific local IgM synthesis usually disappeared by 3 to 6 months after therapy, whereas specific IgG synthesis persisted after recovery. Even in patients with a severely altered blood-brain barrier, the assay discriminated between intrathecal antibody synthesis and antibody leakage from serum. The assay makes diagnostic measurement of B. burgdorferi -specific intrathecal antibody synthesis reliable, rapid, and accessible as a routine serological test.

9/AB/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07426881 91339861 PMID: 1874248
Diagnosis of Lyme borreliosis by an enzyme immunoassay detecting immunoglobulin G reactive to purified Borrelia burgdorferi cell components.
Bergstrom S; Sjostedt A; Dotevall L; Kaijser B; Ekstrand-Hammarstrom B; Wallberg C; Skogman G; Barbour AG
Department of Microbiology, University of Umea, Sweden.
European journal of clinical microbiology & infectious diseases (GERMANY) May 1991 , 10 (5) p422-7, ISSN 0934-9723 Journal Code: EM5
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
An enzyme immunoassay (EIA) developed for the diagnosis of Lyme borreliosis was tested for its specificity and sensitivity in detecting IgG antibodies in patients at various stages of the disease. The EIA is based on a detergent extract of Borrelia burgdorferi which contains 12 proteins of defined molecular weights from Borrelia burgdorferi . The assay showed a specificity of 100% in control sera from 64 healthy individuals, using a cut-off optical density value of 0.13 (means +2- 3 SD). The sensitivity was 100% using sera from 22 Swedish patients with late stage Lyme borreliosis and 43% using sera from 30 patients with the initial stage of the disease. The reactivity of the sera against whole cell preparations, the outer surface proteins OspA and OspB, and the flagella of Borrelia burgdorferi was also tested and compared with the EIA. No cross-reactivity with treponemal antigens was observed when using the EIA.

9/AB/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07372138 91131773 PMID: 1993753
Improved immunoglobulin M serodiagnosis in Lyme borreliosis by using a mu-capture enzyme-linked immunosorbent assay with biotinylated Borrelia burgdorferi flagella .
Hansen K; Pii K; Lebech AM
Department of Infection Immunology, Statens Seruminstitut, Copenhagen, Denmark.

Journal of clinical microbiology (UNITED STATES) Jan 1991 , 29 (1) p166-73, ISSN 0095-1137 Journal Code: HSH
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

A mu-capture enzyme-linked immunosorbent assay (ELISA) for detection of serum immunoglobulin M (IgM) antibodies to Borrelia burgdorferi by using biotinylated purified B. burgdorferi flagella was developed. The diagnostic performance of the mu-capture ELISA was compared with that of a conventional indirect ELISA. Sera from untreated patients with erythema migrans (n = 50), neuroborreliosis (n = 100), and acrodermatitis chronica atrophicans (ACA; n = 48) were investigated. The cutoff of the ELISAs was adjusted to a diagnostic specificity of 98% on the basis of examination of 200 serum specimens from healthy controls. The mu-capture ELISA increased the diagnostic sensitivity in patients with erythema migrans from 32 to 48% (P less than 0.01) and in patients with neuroborreliosis from 37 to 57% (P less than 0.001). Because of an increased signal/noise ratio, the mu-capture ELISA yielded a significantly better quantitative discrimination of individual positive measurements from the cutoff (P less than 0.001). The increased signal/noise ratio was most likely a consequence of the elimination of IgG competition for the test antigen. This may also explain why 12% of patients with ACA showed significantly increased specific IgM levels only by the mu-capture ELISA. Of patients with ACA, 27% had IgM rheumatoid factor. The mu-capture principle with a directly labeled antigen showed no interference with IgM rheumatoid factor, in contrast to the indirect ELISA. The high diagnostic performance and ease of this three-step mu-capture ELISA make it suitable for routine anti-B. burgdorferi IgM serodiagnosis .

9/AB/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07149130 94069040 PMID: 7504314
Cross-reactive antigenic domains of the flagellin protein of Borrelia burgdorferi.
Luft BJ; Dunn JJ; Dattwyler RJ; Gorgone G; Gorevic PD; Schubach WH
Department of Medicine, State University of New York, Stony Brook 11794.
Research in microbiology (FRANCE) May 1993 , 144 (4) p251-7, ISSN 0923-2508 Journal Code: R6F
Contract/Grant No.: RO1 A132454, PHS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

The p41 flagellin of Borrelia burgdorferi is the most common antigen recognized by serum of patients with Lyme borreliosis. This antigen shares amino acid homology, particularly in the amino and carboxy termini, with periflagellar antigens found in other microorganisms including Treponema pallidum. We cloned and expressed the p41 open reading frame in Escherichia coli and expressed it both as TrpE fusion and full-length unfused proteins. Also, we generated deletion constructs of various portions of the gene . Sera from patients with late Lyme borreliosis and secondary syphilis were used to identify the recombinant proteins by immunoblot analysis. Sera from 26 patients with Lyme borreliosis, 20 with secondary syphilis and 10 controls were used to identify cross-reactive domains of the B. burgdorferi flagellin. The variable region (amino acids 131-234) of the protein was recognized by 59% (15/26) of patients with late Lyme borreliosis compared to 30% (6/20) of patients with secondary syphilis and no (0/ 10) control patients. It appears that cross-reactive epitopes between B. burgdorferi and T . pallidum extend to the variable region of the flagellin.

9/AB/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06923228 92194300 PMID: 1372363
Identification of an endoflagellar associated protein in Borrelia burgdorferi.
Eiffert H; Schlott T; Hoppert M; Lotter H; Thomssen R
Department of Medical Microbiology, University of Gottingen, Germany.
Journal of medical microbiology (ENGLAND) Mar 1992 , 36 (3) p209-14 , ISSN 0022-2615 Journal Code: J2N
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
DNA of Borrelia burgdorferi was cleaved by the endonuclease EcoRI and ligated with the bacteriophage expression vector lambda gt11. After infection of the Escherichia coli strain Y1089, the plaques of recombinant phages were screened with a B. burgdorferi antiserum (human) for fusion proteins containing borrelia antigen.s A positive clone produced a hybrid protein (p200) of c. 200 Kda. The corresponding native borrelia protein (p97) was identified as having an Mr of 97 Kda. To localise protein p97 in the B. burgdorferi cell, immunoelectronmicroscopy and a Western blot of isolated flagella were used. Antibodies directed against proteins p200 and p97 recognised epitopes associated with the flagella .

9/AB/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06774428 92015834 PMID: 1920889
Serodiagnosis of Lyme disease by ELISA using Borrelia burgdorferi flagellum antigen]
Kawabata M; Kubo N; Arashima Y; Yoshida M; Kawano K
Department of Clinical Pathology, Nihon University School of Medicine, Tokyo.
Rinsho byori (JAPAN) Aug 1991 , 39 (8) p891-4, ISSN 0047-1860 Journal Code: KIV
Languages: JAPANESE
Document type: Journal Article
Record type: Completed
Antibodies to a 41,000 (41 kD) polypeptide in flagella of Borrelia burgdorferi were measured in patients with Lyme disease in Japan by flagellum ELISA. The IgG and IgM Classes of antibodies to a flagellum antigens were detected in the sera as early as 0.5 months after infection. The IgG antibodies continued to exist in their sera for more than one year, while the IgM antibodies quickly faded out from their sera. With respect to a diagnostic specificity of the flagellum ELISA, false positive reactions showing more than 10% were observed in sera with high levels of IgG or IgM, and with anti-syphilis antibody. This method, however, was unaffected by sera with high levels of IgA, rheumatoid factor or anti-nuclear antibody. In three cases of patients with erythema migrans preceded by tick-bite, and treated with antibiotics, seronegative results were observed by a immunoperoxidase (IP) test. Since two of them showed the positive level of IgM antibody by the flagellum ELISA, this method seems to be more sensitive and useful than the IP test for serodiagnosis of the Lyme disease.

9/AB/23 (Item 23 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06771274 92010250 PMID: 1917048
Preliminary characterization of Borrelia burgdorferi CSF isolates.
Khanakah G; Millner MM; Mullegger RR; Stanek G
Hygiene-Institut der Universitat Wien, Austria.
Infection (GERMANY) Jul-Aug 1991 , 19 (4) p287-8, ISSN 0300-8126
Journal Code: GO8
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
Borrelia burgdorferi was cultivated from three cerebrospinal fluid (CSF) samples of children (aged three and a half, four and a half and eight years) who were admitted to the hospital because of acute facial palsy, aseptic meningitis, and aseptic meningitis plus facial palsy. CSF was taken on day one in two cases and on day two in the remaining case after onset of symptoms. All three strains showed a very similar SDS-PAGE pattern, without an OspB and 20kD band. However, of nine monoclonal antibodies (Moab) raised against B. burgdorferi B31, the Moab H5332 recognized two strains, one of them very weakly, and the flagella specific Moabs H9724, H605, and H6TS (less intensively) recognized all strains. This preliminary characterization reveals heterogeneity among CSF Borrelia isolates of cases from a very close geographic area.

9/AB/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06629675 90251638 PMID: 2339119
Monoclonal antibodies specific for the outer surface protein A (OspA) of Borrelia burgdorferi prevent Lyme borreliosis in severe combined immunodeficiency (scid) mice.
Schaible UE; Kramer MD; Eichmann K; Modolell M; Museteanu C; Simon MM
Max-Planck-Institut fur Immunbiologie, Freiburg, Federal Republic of Germany.
Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) May 1990 , 87 (10) p3768-72, ISSN 0027-8424
Journal Code: PV3
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
We have recently shown that viable Borrelia burgdorferi organisms induce a chronic infection associated with arthritis and carditis in severe combined immunodeficiency (scid) mice but not in immunocompetent mice. The disease is similar to that found in patients suffering from Lyme disease. We now show that B. burgdorferi -specific immune mouse sera as well as a monoclonal antibody to the spirochetal outer surface antigen A (31 kDa) but not monoclonal antibodies specific for the 41-kDa antigenic component of the periplasmic flagella are able to prevent (or mitigate) the development of the disease in scid mice when passively transferred at the time of the bacterial inoculation. The identification of a B. burgdorferi -associated protective antigen suggests that the corresponding spirochetal protein should be tested as a vaccine against Lyme disease.

9/AB/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06585317 89247122 PMID: 2719854
Characterization of the first tick isolate of Borrelia burgdorferi from Italy.
Cinco M; Banfi E; Trevisan G; Stanek G
Istituto di Microbiologia, Universita di Trieste, Italy.

APMIS (DENMARK) Apr 1989 , 97 (4) p381-2, ISSN 0903-4641
Journal Code: AMS
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
We report on the first isolation of a spirochetal organism from Ixodes ricinus ticks of the Trieste area (Northern Italy) which was identified as Borrelia burgdorferi by its reactivity with specific monoclonal antibodies directed against the OSPA and flagella proteins.

9/AB/26 (Item 26 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06581441 89234551 PMID: 2715325
Serodiagnosis of erythema migrans and acrodermatitis chronica atrophicans by the Borrelia burgdorferi flagellum enzyme-linked immunosorbent assay.
Hansen K; Asbrink E
Department of Treponematoses, Statens Seruminstitut, Copenhagen, Denmark.
Journal of clinical microbiology (UNITED STATES) Mar 1989 , 27 (3) p545-51, ISSN 0095-1137 Journal Code: HSH
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The diagnostic performance of an enzyme-linked immunosorbent assay (ELISA) using purified Borrelia burgdorferi flagella as test antigen was compared with that of a B. burgdorferi sonic extract ELISA. We tested sera from 200 healthy controls, 107 patients with erythema migrans (EM), 50 patients with acrodermatitis chronica atrophicans (ACA), and 98 patients with various dermatological disorders without clinical evidence of active Lyme borreliosis. The flagellum ELISA was significantly more sensitive than the sonic extract ELISA. With sera from patients with EM, the diagnostic sensitivity for immunoglobulin G (IgG) antibody detection increased from 11.2 to 35.5% (P less than 0.001) and for IgM antibody detection it increased from 16.6 to 44.8% (P less than 0.001). In the flagellum ELISA, the number of positive tests increased significantly (P less than 0.005) when the duration of EM exceeded 1 month, but still only about 50% of patients with longstanding (1 to 12 months) untreated EM were IgG seropositive. Concomitant general symptoms did not affect the antibody level, whereas patients with multiple erythema were more frequently seropositive. All sera from patients with EM which were positive in the sonic extract ELISA were also positive in the flagellum ELISA. Not only did the overall number of positive tests increase, but the flagellum ELISA yielded a significantly better quantitative discrimination between seropositive patients and controls (P less than 0.002). IgG antibodies to the B. burgdorferi flagellum were found in all sera from patients with ACA, indicating persistence of an antiflagellum immune response in late stages of Lyme borreliosis.(ABSTRACT TRUNCATED AT 250 WORDS)

9/AB/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06555162 89109463 PMID: 2913024
Antigenically variable Borrelia burgdorferi isolated from cottontail rabbits and Ixodes dentatus in rural and urban areas.
Anderson JF; Magnarelli LA; LeFebvre RB; Andreadis TG; McAninch JB; Perng GC; Johnson RC
Department of Entomology, Connecticut Agricultural Experiment Station, New Haven 06504.

Journal of clinical microbiology (UNITED STATES) Jan 1989 , 27 (1) p13-20, ISSN 0095-1137 Journal Code: HSH
Contract/Grant No.: AI 18153, AI, NIAID
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

Spirochetes were isolated from 71 subadult Ixodes dentatus removed from cottontail rabbits captured in Millbrook, N.Y., and in New York, N.Y. Spirochetes were also cultured from kidney tissues of six rabbits. While all isolates reacted with monoclonal antibody H9724, which identifies the spirochetes as borreliae, more than half did not bind with antibody H5332 and even fewer reacted with H3TS, both of which were produced to outer surface protein A of Borrelia burgdorferi . Sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein profiles of three isolates differed from one another and from all previously characterized B. burgdorferi strains from humans, ticks, and wildlife in North America. The 12 periplasmic flagella that originated subterminally from each pointed end of a rabbit Borellia isolate contrasted with the 11 or fewer flagella for B. burgdorferi reported previously from North America. Although DNA homology and restriction endonuclease analysis also revealed differences among a rabbit kidney isolate, an I. dentatus isolate, and B. burgdorferi B31, similarities were sufficient to lead us to conclude that the borreliae in rabbits and I. dentatus are B. burgdorferi . Enzyme-linked immunosorbent assay titers of sera from humans with diagnosed Lyme disease to rabbit tick B. burgdorferi were often similar to one another and to those recorded for a reference B. burgdorferi strain.

9/AB/28 (Item 28 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06503275 89102195 PMID: 3213319
First isolation of Borrelia burgdorferi, the agent of Lyme borreliosis, from Ixodes ricinus (Acari: Ixodidae) in Berlin (West).
Schonberg A; Camey C; Kahl O; Wilske B; Preac-Mursic V; Hovind-Hougen K
Institute for Veterinary Medicine, Federal Health Office, Berlin.
Zentralblatt fur Bakteriologie, Mikrobiologie, und Hygiene. Series A, Medical microbiology, infectious diseases, virology, parasitology (GERMANY, WEST) Jun 1988 , 268 (4) p487-94, ISSN 0176-6724 Journal Code: Y55
Languages: ENGLISH
Document type: Journal Article
Record type: Completed

In 1984, two human cases of tick-borne Lyme borreliosis with considerable neurologic involvement were reported in Berlin (West). The diagnosis of Lyme borreliosis was serologically confirmed. The ticks which had transmitted the Borrelia were from Berlin (West). In the autumn of 1985, 156 ticks were collected in forests of Berlin (West) for the cultural detection of spirochetes by using BSK II medium. Three strains of spirochetes were isolated (from a pooled sample of two nymphs, and samples of one nymph and one female tick, respectively). These isolates were identified as Borrelia burgdorferi by means of SDS-PAGE, Western blot (using monoclonal antibody H 5332), microscopic agglutination test and electron microscopy. Investigations with the electron microscope showed that cells of two isolates (strains 2/B45 and 3/B56) had 8 flagella inserted at each end. The cells of the third isolate (strain 1/B29) had 9 flagella inserted at each end. This type had not been observed before.

9/AB/29 (Item 29 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06386246 86194740 PMID: 3516878
A Borrelia-specific monoclonal antibody binds to a flagellar epitope.
Barbour AG; Hayes SF; Heiland RA; Schrumpf ME; Tessier SL
Infection and immunity (UNITED STATES) May 1986 , 52 (2) p549-54, ISSN 0019-9567 Journal Code: GO7
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
In immunofluorescence assays monoclonal antibody H9724 recognized eight species of the spirochetal genus Borrelia but not representatives of the genera Treponema, Leptospira, and Spirochaeta. We examined the reactivity of H9724 against subcellular components of Borrelia hermsii, an agent of relapsing fever, and B. burgdorferi , the cause of Lyme disease. H9724 bound to isolated periplasmic flagella of the two borreliae. In Western blots the antibody reacted with the predominant protein in flagellar preparations from B. hermsii and B. burgdorferi ; the apparent molecular weights of these flagellins were 39,000 and 41,000, respectively.

9/AB/30 (Item 30 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06199927 86044380 PMID: 3903977
Tick-borne Borrelia infection in Sweden.
Stiernstedt G
Scandinavian journal of infectious diseases (SWEDEN) 1985 , 45 p1-70 , ISSN 0300-8878 Journal Code: UCY
Languages: ENGLISH
Document type: Journal Article; Review
Record type: Completed
Spirochetes were cultivated from 17% of 114 Ixodes ricinus ticks in the Stockholm area. Three strains of these spirochetes were selected for studies by electron microscopy. These three strains had definite morphological similarities to spirochetes of the genus Borrelia, as judged by the number of flagella , absence of cytoplasmic tubules, and dimensions. The three strains were not identical , but seemed to consist of two different kinds of cells, one with eight and one with eleven flagella . The three strains were also shown to react with a monoclonal antibody that reacts with Lyme disease spirochetes (Borrelia burgdorferi), but not with strains of other Borreliae, Treponemes, or Leptospiras. These results indicate the possibility of transmission of Borrelia spirochetes from ticks to humans in Sweden. The antibody response to one of the spirochetal strains isolated from Swedish I. ricinus was studied in 37 patients with the typical clinical picture of erythema chronicum migrans (ECM), in 45 patients with chronic meningitis (CMe) cured by high-dose intravenous penicillin, in 298 patients with post-infectious arthritis, and in controls. The antibody response was estimated by indirect immunofluorescence assay (IFA) enzyme-linked immunosorbent assay (ELISA). The antibody levels differed significantly between patients with CMe and healthy individuals (p less than 0.001), both with respect to serum antibody levels and CSF-antibody levels. The antibody levels also differed significantly between patients with ECM and healthy controls as measured by ELISA (p less than 0.05), whereas the difference was not significant as measured by IFA. Five of 298 patients with post-infectious arthritis had higher titers than any of the controls, and two of these five patients had titers higher than any patient with CMe or ECM. These results indicate spirochetal aetiology of ECM, and in some patents with CMe or postinfectious arthritis. As a diagnostic test for ECM, both IFA and ELISA were of limited value, since only 5/37 (14%) ECM patients were positive by IFA, and 14/37 (38%) by ELISA. Regarding patients with CMe,

23/45 (51%) were seropositive by IFA and 30/45 (67%) by ELISA. However, measurement of CSF-antibodies were found to be a more sensitive method than measurement of serum antibodies both by IFA and ELISA, since 38/45 (84%) CMe patients were positive by IFA, and 41/45 (91%) by ELISA. In addition, estimation of CSF antibodies was also found to be a more specific method than estimation of serum antibodies.(ABSTRACT TRUNCATED AT 400 WORDS)

9/AB/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06020125 88139744 PMID: 3343329
Measurement of antibodies to the Borrelia burgdorferi flagellum improves serodiagnosis in Lyme disease.
Hansen K; Hindersson P; Pedersen NS
Borrelia Laboratory, Department of Treponematoses, Statens Seruminstitut, Copenhagen, Denmark.
Journal of clinical microbiology (UNITED STATES) Feb 1988 , 26 (2) p338-46, ISSN 0095-1137 Journal Code: HSH
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The isolation of Borrelia burgdorferi flagella and an enzyme-linked immunosorbent assay (ELISA) for detection of immunoglobulin G (IgG) and IgM to the B. burgdorferi flagellum are described. The diagnostic performance of the flagellum ELISA for serodiagnosis of Lyme disease was compared with the performance of a traditional whole cell B. burgdorferi sonic extract ELISA. We examined sera and cerebrospinal fluid (CSF) from 56 patients with lymphocytic meningoradiculitis (Bannwarth's syndrome), the most frequent secondary-stage manifestation of Lyme disease in Europe. Two hundred healthy individuals and patients with aseptic meningitis, encephalitis, Guillain-Barre syndrome, and syphilis served as controls. The flagellum ELISA was significantly more sensitive than the sonic extract ELISA. The diagnostic sensitivities were increased from 41.1 to 76.8% (P less than 0.01) for IgG and from 35.7 to 67.9% (P less than 0.05) for IgM detection in serum. The increase in sensitivity was most pronounced in patients with a short duration of disease (less than 20 days after onset). The diagnostic specificity increased for IgG detection but was almost unaltered for IgM. The flagellum ELISA did not improve the diagnostic sensitivity of measuring antibodies to borreliae in CSF, most likely owing to the low level of unspecific antibodies in CSF compared with serum. The cross-reactivity of sera and CSF from patients with syphilis decreased significantly. The flagellum antigen of B. burgdorferi shows no strain variation, is easy to purify in sufficient quantity, and is therefore a suitable reference antigen for routine serodiagnosis of Lyme disease.

9/AB/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05581740 90103448 PMID: 2481425
Molecular mimicry and Lyme borreliosis: a shared antigenic determinant between Borrelia burgdorferi and human tissue.
Aberer E; Brunner C; Suchanek G; Klade H; Barbour A; Stanek G; Lassmann H
Department of Dermatology II, University of Vienna, Austria.
Annals of neurology (UNITED STATES) Dec 1989 , 26 (6) p732-7, ISSN 0364-5134 Journal Code: 6AE
Contract/Grant No.: AI24424, AI, NIAID
Comment in Ann Neurol. 1990 Aug;28(2) 195-6
Languages: ENGLISH
Document type: Journal Article

Record type: Completed
The pathogenesis of chronic manifestations in Lyme borreliosis, a disease induced by Borrelia burgdorferi , is at present unresolved. By testing monoclonal antibodies directed against various borrelia antigens, we found an antigenic determinant shared by the 41 kDa flagella protein and human tissue, especially prominent on myelinated fibers of human peripheral nerve, on nerve cells and axons of the central nervous system, as well as on certain epithelial cells (including joint synovia) and on heart muscle cells. Immune reactions against such a shared antigen could play a pathogenetic role in chronic organ manifestations of Lyme borreliosis.

9/AB/33 (Item 33 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05419781 90006687 PMID: 2676703
N-terminal amino acid sequence of the Borrelia burgdorferi flagellin.
Gassmann GS; Deutzmann R; Vogt A; Gobel UB
Institut fur Medizinische Mikrobiologie und Hygiene, Freiburg, F.R.G.
FEMS microbiology letters (NETHERLANDS) Jul 1 1989 , 51 (1) p101-5, ISSN 0378-1097 Journal Code: FML
Languages: ENGLISH
Document type: Journal Article
Record type: Completed
The 41 kDa flagellar protein of Borrelia burgdorferi appears to be an immunodominant antigen producing an early and strong response in most, if not all, individuals during infection in humans. It would represent a very good antigen for serodiagnosis of Lyme disease, if its crossreactivity with flagella of other bacteria was low. To gain information on this point we isolated the B. burgdorferi flagellin by preparative two-dimensional electrophoresis for N-terminal amino acid analysis. By comparing the N-terminal amino acid sequences of flagellar proteins from other eubacteria we found that the first six out of twenty nine amino acids were identical to the Treponema pallidum and Treponema phagedenis 'class B' flagellins. All 29 N-terminal residues exhibited a moderate inter-genus homology (44-55%), in contrast to the high degree (67-95%) of inter-species conservation of the treponemal 'class B' flagellar N-terminal sequences. There was little similarity to other flagellins except the B. subtilis flagellar protein.

9/AB/34 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07744659 BIOSIS NO.: 000041053455
ISOLATION AND CHARACTERIZATION OF A FLAGELLA -LESS BORRELIA- BURGDORFERI
AUTHOR: BUNDOC V G; HOLT S E; BARBOUR A G
AUTHOR ADDRESS: UNIV. TEX. HEALTH SCI. CENT., SAN ANTONIO, TEX., USA.
JOURNAL: 91ST GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY 1991, DALLAS, TEXAS, USA, MAY 5-9, 1991. ABSTR GEN MEET AM SOC MICROBIOL 91 (0). 1991. 66. 1991
CODEN: AGMME
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH
1991

9/AB/35 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07478005 BIOSIS NO.: 000091062724
IMPROVED IMMUNOGLOBULIN M SERODIAGNOSIS IN LYME BORRELIOSIS BY USING A MU-CAPTURE ELISA WITH BIOTINYLATED BORRELIA- BURGDORFERI FLAGELLA
AUTHOR: HANSEN K; PII K; LEBECH A-M
AUTHOR ADDRESS: BORRELIA LAB., DEP. INFECTION IMMUNOL. STATENS SERUMINSTITUT, COPENHAGEN, DENMARK.
JOURNAL: J CLIN MICROBIOL 29 (1). 1991. 166-173. 1991
FULL JOURNAL NAME: Journal of Clinical Microbiology
CODEN: JCMID
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A .mu.-capture enzyme-linked immunosorbent assay (ELISA) for detection of serum immunoglobulin M (IgM) antibodies to Borrelia burgdorferi by using biotinylated purified B. burgdorferi flagella , was developed. The diagnostic performance of the .mu.-capture ELISA was compared with that of a conventional indirect ELISA. Sera from untreated patients with erythema migrans (n = 50), neuroborreliosis (n = 100), and acrodermatitis chronica atrophicans (ACA; n= 48) were investigated. The cutoff of the ELISAs was adjusted to a diagnostic specificity of 98% on the basis of examination of 200 serum specimens from healthy controls. The .mu.-capture ELISA increased the diagnostic sensitivity in patients with erythema migrans from 32 to 48% (P < 0.01) and in patients with neuroborreliosis from 37 to 57% (P < 0.001). Because of an increased signal/noise ratio, the .mu.-capture ELISA yielded a significantly better quantitative discrimination of individual positive measurements from the cutoff (P < 0.001). The increased signal/noise ratio was most likely a consequence of the elimination of IgG competition for the test antigen. This may also explain why 12% of patients with ACA showed significantly increased specific IgM levels only by the .mu.-capture ELISA. Of patients with ACA, 27% had IgM rheumatoid factor. The .mu.-capture principle with a directly labeled antigen showed no interference with IgM rheumatoid factor, in contrast to the indirect ELISA. The high diagnostic performance and ease of this three-step .mu.-capture ELISA make it suitable for routine anti-B. burgdorferi IgM serodiagnosis .

1991

9/AB/36 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

07477799 BIOSIS NO.: 000091062518
SENSITIVE DETECTION OF TREPONEMA-PALLIDUM BY USING THE POLYMERASE CHAIN REACTION
AUTHOR: BURSTAIN J M; GRIMPREL E; LUKEHART S A; NORGARD M V; RADOLF J D
AUTHOR ADDRESS: DEP. INTERN. MED., UNIV. TEXAS SOUTHWESTERN MEDICAL CENT. DALLAS, DALLAS, TEX. 75235.
JOURNAL: J CLIN MICROBIOL 29 (1). 1991. 62-69. 1991
FULL JOURNAL NAME: Journal of Clinical Microbiology
CODEN: JCMID
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: We have developed a sensitive assay for Treponema pallidum subsp. pallidum (T . pallidum), the agent of venereal syphilis, based upon the polymerase chain reaction (PCR). A 658-bp portion of the gene encoding the 47-kDa membrane immunogen was amplified, and the PCR products were

probed by DNA-DNA hybridization with a 496-bp fragment internal to the amplified DNA. The assay detected approximately 0.01 pg of purified T . pallidum DNA, and positive results were obtained routinely from suspensions of treponemes calculated to contain 10 or more organisms and from some suspensions calculated to contain a single organism. Specific PCR products were obtained for the closely related agent of yaws, Treponema pallidum subsp. pertenue, but not with human DNA or DNAs from other spirochetes (including Borrelia burgdorferi), skin microorganisms, sexually transmitted disease pathogens, and central nervous system pathogens. T . pallidum DNA was detected in serum, cerebrospinal fluids, and amniotic fluids from syphilis patients but not in nonsyphilitic controls. T . pallidum DNA was also amplified from paraffin-embedded tissue. The diagnosis of syphilis by using PCR may become a significant addition to the diagnostic armamentarium and a valuable technique for the investigation of syphilis pathogenesis.

1991

9/AB/37 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

06651942 BIOSIS NO.: 000087094119
SERODIAGNOSIS OF ERYTHEMA MIGRANS AND ACRODERMATITIS CHRONICA ATROPHICANS BY THE BORRELIA-BURGDORFERI FLAGELLUM ELISA
AUTHOR: HANSEN K; ASBRINK E
AUTHOR ADDRESS: BORRELIA LAB., DEP. TREPONEMATOSES, STATENS SERUMINSTITUT, 2300 COPENHAGEN S., DENMARK.
JOURNAL: J CLIN MICROBIOL 27 (3). 1989. 545-551. 1989
FULL JOURNAL NAME: Journal of Clinical Microbiology
CODEN: JCMID
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The diagnostic performance of an enzyme-linked immunosorbent assay (ELISA) using purified Borrelia burgdorferi flagella as test antigen was compared with that of a B. burgdorferi sonic extract ELISA. We tested sera from 200 healthy controls, 107 patients with erythema migrans (EM), 50 patients with acrodermatitis chronica atrophicans (ACA), and 98 patients with various dermatological disorders without clinical evidence of active Lyme borreliosis. The flagellum ELISA was significantly more sensitive than the sonic extract ELISA. With sera from patients with EM, the diagnostic sensitivity for immunologlobulin G (IgG) antibody detection increased from 11.2 to 35.5% ($P < 0.001$) and for IgM antibody detection it increased from 16.6 to 44.8% ($P < 0.001$). In the flagellum ELISA, the number of positive tests increased significantly ($P < 0.005$) when the duration of EM exceeded 1 month, but still only about 50% of patients with longstanding (1 to 12 months) untreated EM were IgG seropositive. Concomitant general symptoms did not affect the antibody level, whereas patients with multiple erythema were more frequently seropositive. All sera from patients with EM which were positive in the sonic extract ELISA were also positive in the flagellum ELISA. Not only did the overall number of positive tests increase, but the flagellum ELISA yielded a significantly better quantitative discrimination between seropositive patients and controls ($P < 0.002$). IgG antibodies to the B. burgdorferi flagellum were found in all sera from patients with ACA, indicating persistence of an antiflagellum immune response in late stages of Lyme borreliosis. IgM reactivity in sera from patients with ACA was shown to be nonspecific and the result of IgM rheumatoid factor. A rheumatoid factor was detected in sera from 32% of

patients with ACA, compared with 7.5% of patients with EM. The improved diagnostic performance, the ease of standardization of the flagellum antigen, and the lack of strain variation make the B. burgdorferi flagellum a needed reference antigen for growing routine serology in Lyme borreliosis.

1989

9/AB/38 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

05825003 Genuine Article#: WZ944 Number of References: 58
Title: The flgK motility operon of Borrelia burgdorferi is initiated by a sigma(70)-like promoter (ABSTRACT AVAILABLE)
Author(s): Ge YG; Old IG; SaintGirons I; Charon NW (REPRINT)
Corporate Source: W VIRGINIA UNIV,HLTH SCI CTR, DEPT MICROBIOL, BOX 9177/MORGANTOWN//WV/26506 (REPRINT); W VIRGINIA UNIV,HLTH SCI CTR, DEPT MICROBIOL/MORGANTOWN//WV/26506; INST PASTEUR,UNITE BACTERIOL MOL & MED/F-75724 PARIS 15//FRANCE/
Journal: MICROBIOLOGY-UK, 1997 , V143, 5 (MAY), P1681-1690
ISSN: 1350-0872 Publication date: 19970500
Publisher: SOC GENERAL MICROBIOLOGY, HARVEST HOUSE 62 LONDON ROAD, READING, BERKS, ENGLAND RG1 5AS
Language: English Document Type: ARTICLE
Abstract: A duster of flagellar genes of Borrelia burgdorferi was identified and sequenced. This cluster comprises an operon, designated the flgK operon, which is initiated by a sigma(70)-like promoter. The flgK operon consists of flbF (function unknown), flgK (encoding HAP1), flgL (encoding HAP3) and orfX (function unknown), and maps at 185 kb on the chromosome. In other bacteria, the hook-associated proteins HAP1 and HAP3 connect the flagellar filament to the hook and are required for the last stage of flagellar assembly. Reverse transcriptase-PCR analysis indicated that flbF through to orfX are transcribed as a single mRNA, and primer extension analysis revealed that transcription of the flgK operon is initiated by a sigma(70)-like promoter upstream of flbF. Subcloning the flgK promoter element into a promoter probe cat vector revealed that the flgK promoter element had strong activity in both Escherichia coli and Salmonella typhimurium. In addition, when this construct was transformed into a fliA mutant of 5, typhimurium which lacked a functional flagellar-specific sigma(28) factor, the flgK promoter was still functional. Based on these results, the promoter element of the flagellin gene (fla, hereafter referred to as flag) was re-examined, flag encodes the flagellar filament protein, and a sigma(gp33-34)-like promoter has been reported to be involved in the transcription of this gene. A transcriptional start point was found 1 bp downstream of the reported start site. The sequence around -10 and -35 are consistent with the presence of a sigma(70)-like promoter in addition to the putative sigma(gp33-34)-like promoter for flag. In contrast to the flgK promoter element, no activity was detected after subcloning a flaB promoter element into the promoter probe cat vector. Because a sigma(70)-like promoter rather than a unique flagellar sigma factor is involved in the later stage of flagellar assembly, the regulation of B. burgdorferi flagellar genes is evidently different from that of other bacteria.

9/AB/39 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

02147025 Genuine Article#: KE540 Number of References: 37
Title: BACTERIAL FLAGELLAR DIVERSITY AND SIGNIFICANCE IN PATHOGENESIS (Abstract Available)
Author(s): PENN CW; LUKE CJ
Corporate Source: UNIV BIRMINGHAM,SCH BIOL SCI/BIRMINGHAM B15 2TT/W MIDLANDS/ENGLAND/
Journal: FEMS MICROBIOLOGY LETTERS, 1992 , V100, N1-3 (DEC 15), P331-336
ISSN: 0378-1097
Language: ENGLISH Document Type: ARTICLE
Abstract: Bacterial flagella are structurally diverse, ranging from the thoroughly investigated model examples found in Escherichia coli and Salmonella typhimurium to the more exotic sheathed flagella of, for example, Helicobacter pylori, and the complex multi-flagellin endoflagella found in many spirochaetes. We summarize some of the emerging structural and genetic findings relating to these more novel flagellar types, and outline their possible significance in the pathogenicity of some medically important bacteria.

9/AB/40 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

01156759 Genuine Article#: GA133 Number of References: 29
Title: LYME NEUROBORRELIOSIS - A NEW SENSITIVE DIAGNOSTIC ASSAY FOR INTRATHECAL SYNTHESIS OF BORRELIA-BURGDORFERI-SPECIFIC IMMUNOGLOBULIN-G, IMMUNOGLOBULIN-A, AND IMMUNOGLOBULIN-M (Abstract Available)
Author(s): HANSEN K; LEBECH AM
Corporate Source: STATENS SERUM INST,DEPT INFECT IMMUNOL,BORRELIALAB,ARTILLERIVEJ 5/DK-2300 COPENHAGEN//DENMARK/
Journal: ANNALS OF NEUROLOGY, 1991 , V30, N2, P197-205
Language: ENGLISH Document Type: ARTICLE
Abstract: An antibody capture enzyme-linked immunosorbent assay was developed to measure directly intrathecal immunoglobulin (Ig) G, A, and M synthesis specific for Borrelia burgdorferi . Purified, biotin-avidin-peroxidase-labeled B. burgdorferi flagella was used as test antigen. Paired cerebrospinal fluid and serum specimens from 100 patients with clinically definite neuroborreliosis and 35 control subjects with neurological diseases were examined. Significant B. burgdorferi -specific intrathecal IgG, A, and M production was found in 89%, 65%, and 67% of patients with neuroborreliosis. Local synthesis of specific IgA was only seen in patients with significant local IgG synthesis. Antibody production in cerebrospinal fluid began by 2 weeks after onset of neurological symptoms. At the end of the second week specific IgM, IgG, or both, was detected in 88% of the patients. Specific IgG synthesis was present in all patients by 6 weeks after onset. Specific local IgM synthesis usually disappeared by 3 to 6 months after therapy, whereas specific IgG synthesis persisted after recovery. Even in patients with a severely altered blood-brain barrier, the assay discriminated between intrathecal antibody synthesis and antibody leakage from serum. The assay makes diagnostic measurement of B. burgdorferi -specific intrathecal antibody synthesis reliable, rapid, and accessible as a routine serological test.

9/AB/41 (Item 4 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

00708076 Genuine Article#: EP044 Number of References: 32
Title: IMPROVED IMMUNOGLOBULIN-M SERODIAGNOSIS IN LYME BORRELIOSIS BY USING A MU-CAPTURE ENZYME-LINKED-IMMUNOSORBENT-ASSAY WITH BIOTINYLATED BORRELIA- BURGDORFERI FLAGELLA (Abstract Available)
Author(s): HANSEN K; PII K; LEBECH AM
Corporate Source: STATENS SERUM INST,DEPT INFECT IMMUNOL,BORRELIALAB/DK-2300 COPENHAGEN//DENMARK/; DAKOPATTS/COPENHAGEN//DENMARK/
Journal: JOURNAL OF CLINICAL MICROBIOLOGY, 1991 , V29, N1, P166-173
Language: ENGLISH Document Type: ARTICLE
Abstract: A mu-capture enzyme-linked immunosorbent assay (ELISA) for detection of serum immunoglobulin M (IgM) antibodies to Borrelia burgdorferi by using biotinylated purified B. burgdorferi flagella was developed. The diagnostic performance of the mu-capture ELISA was compared with that of a conventional indirect ELISA. Sera from untreated patients with erythema migrans (n = 50), neuroborreliosis (n = 100), and acrodermatitis chronica atrophicans (ACA; n = 48) were investigated. The cutoff of the ELISAs was adjusted to a diagnostic specificity of 98% on the basis of examination of 200 serum specimens from healthy controls. The mu-capture ELISA increased the diagnostic sensitivity in patients with erythema migrans from 32 to 48% ($P < 0.01$) and in patients with neuroborreliosis from 37 to 57% ($P < 0.001$). Because of an increased signal/noise ratio, the mu-capture ELISA yielded a significantly better quantitative discrimination of individual positive measurements from the cutoff ($P < 0.001$). The increased signal/noise ratio was most likely a consequence of the elimination of IgG competition for the test antigen. This may also explain why 12% of patients with ACA showed significantly increased specific IgM levels only by the mu-capture ELISA. Of patients with ACA, 27% had IgM rheumatoid factor. The mu-capture principle with a directly labeled antigen showed no interference with IgM rheumatoid factor, in contrast to the indirect ELISA. THE high diagnostic performance and ease of this three-step mu-capture ELISA make it suitable for routine anti-B. burgdorferi IgM serodiagnosis .

9/AB/42 (Item 1 from file: 35)
DIALOG(R)File 35:Dissertation Abs Online

01556734 AAD9716360
MOLECULAR ANALYSES OF MOTILITY AND CHEMOTAXIS GENES AND THEIR ORGANIZATION IN BORRELIA BURGDORFERI (FLAB, FLGE, TREPONEMA, BACILLUS SUBTILIS)
Author: GE, YIGONG
Degree: PH.D.
Year: 1996
Corporate Source/Institution: WEST VIRGINIA UNIVERSITY (0256)
Source: VOLUME 57/12-B OF DISSERTATION ABSTRACTS INTERNATIONAL. PAGE 7360. 271 PAGES

Motility and chemotaxis have been considered as important candidates for the study of virulence in many pathogenic spirochetes, including B. burgdorferi . Little is known about the underlying mechanisms of motility and chemotaxis. In this study, we have carried out an extensive search for the motility and chemotaxis genes in B. burgdorferi . The unique technique of "semi-random PCR chromosome walking" was developed and used to clone several large motility and chemotaxis gene clusters. Together with the previously reported flaB and flgE genes, 38 motility and chemotaxis related genes (more than 36 kb) were identified . These genes demonstrated extensive homology with their bacterial counterparts, especially with those from Treponema and Bacillus subtilis. Motility and chemotaxis genes

constitute four major clusters on the chromosome of B. burgdorferi . Transcript analyses using RT-PCR and primer extension revealed that at least five operons were present in these motility and chemotaxis clusters; they were flgB, flgK, flaB, flaA /che, and fliD operons. The conserved $\sigma\sp{70}$-like promoters were involved in all of these operons, which is distinct to those of other bacteria. These results indicate that although the motility and chemotaxis genes in B. burgdorferi are well conserved, the transcriptional regulation of flagellar gene expression in B. burgdorferi is different from those of other bacteria. To further analyze these motility and chemotaxis genes, we overexpressed several motility and chemotaxis proteins in E. coli. Serological analyses using recombinant motility and chemotaxis proteins (FlgE, FliG, FliI, CheY and FlaA) indicate that except for FlgB and FlgE, these proteins were not immunodominant antigens during infection. Thus, they are not good candidates for diagnostic purposes. In order to further study the functions of these genes, we made an extensive effort to develop a gene inactivation system. Several different antibiotic constructs (gyrB-coumermycin resistant gene, cat and kanamycin resistant genes) were inserted into several motility genes. Unfortunately, so far none of these constructs worked successfully in B. burgdorferi . This study is the first important step toward the achievement of a better understanding of the genetics of spirochetal motility and chemotaxis.

9/AB/43 (Item 1 from file: 50)
DIALOG(R)File 50:CAB Abstracts

02797203 CAB Accession Number: 940800178
Determination of antibody titres for Borrelia burgdorferi in the serum of gypsies living in Attika, Greece.
Chatzipanagiotou, S.; Papandreou-Rakitzis, P.; Malamou-Ladas, H.; Antoniou, P.
Department of Clinical Microbiology, Pendeli Children's Hospital, Pendeli 15236, Greece.
European Journal of Clinical Microbiology & Infectious Diseases vol. 11 (5): p.477-478
Publication Year: 1992
ISSN: 0934-9723 --
Language: English
Document Type: Journal article
Serum (271 samples) from gypsies residing in different parts of Attika (Athens metropolitan area), Greece, were tested for the presence of specific antibodies to soluble antigens of the B31 strain of B. burgdorferi . Tests were performed using a commercially available enzyme immunoassay kit. Results showed that 3 (1.11%) samples were positive, 2 (0.74%) were equivocal, and 266 (98.15%) were negative. Western blot analysis of the 2 equivocal sera showed a positive reaction of both with the 41kDa major flagella antigen only. 6 ref.

9/AB/44 (Item 2 from file: 50)
DIALOG(R)File 50:CAB Abstracts

02679792 CAB Accession Number: 930514983
Comparison of four serological tests for Borrelia burgdorferi in Bell's palsy.
Lakos, A.
Central Hospital for Infectious Diseases, PO Box 29, H-1450 Budapest, Hungary.

Serodiagnosis and Immunotherapy in Infectious Disease vol. 4 (4): p.271-275
Publication Year: 1990
ISSN: 0888-0786 --
Language: English
Document Type: Journal article
Four serological tests for B. burgdorferi were compared in 117 Bell's palsy patients. Sera were tested against a whole cell sonicate by ELISA (SELISA), against a flagella extract by ELISA (FELISA), traditional IFA and IFA after absorption with Treponema reiteri (ABIF). Altogether 35, 35, 29 and 20 cases were positive by IFA, FELISA, ABIF and SELISA, respectively. All 10 typical Bannwarth's syndrome cases were positive by IFA and ABIF, 9 of FELISA, but only 6 by SELISA. In the 16 patients with concomitant erythema chronicum migrans (ECM), 15 proved to be positive by IFA, 12 by FELISA, 11 by ABIF and 7 by SELISA. This data show that IFA may be one of the best routine laboratory indicators for borrelial infection. 7 ref.

9/AB/45 (Item 1 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01621874 SUPPLIER NUMBER: 18403838 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Differential responsiveness to interferon-alpha in beta-cells and non-beta cells.
Bonnevie-Nielsen, V.; Buschard, K.; Dyrberg, T.
Diabetes, v45, n6, p818(4)
June,
1996
PUBLICATION FORMAT: Magazine/Journal ISSN: 0012-1797 LANGUAGE: English
RECORD TYPE: Fulltext TARGET AUDIENCE: Professional
WORD COUNT: 2722 LINE COUNT: 00226

9/AB/46 (Item 2 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01484204 SUPPLIER NUMBER: 15537476 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Legionnaires' disease.
Roig, Jorge; Domingo, Christian; Morera, Jose
Chest, v105, n6, p1817(9)
June,
1994
PUBLICATION FORMAT: Magazine/Journal ISSN: 0012-3692 LANGUAGE: English
RECORD TYPE: Fulltext TARGET AUDIENCE: Professional
WORD COUNT: 8262 LINE COUNT: 00707

9/AB/47 (Item 3 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01483437 SUPPLIER NUMBER: 15488742 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Safety and immunogenicity of a recombinant outer surface protein A Lyme vaccine.
Keller, David; Koster, Frederick T.; Marks, Donald H.; Hosbach, Philip; Erdile, Lorne F.; Mays, John P.
JAMA, The Journal of the American Medical Association, v271, n22, p1764(5)
June 8,

1994
PUBLICATION FORMAT: Magazine/Journal ISSN: 0098-7484 LANGUAGE: English
RECORD TYPE: Fulltext; Abstract TARGET AUDIENCE: Professional
WORD COUNT: 4383 LINE COUNT: 00365

ABSTRACT: Recombinant outer surface lipoprotein A (OspA) Lyme vaccine appears safe and effective in protecting adults from Lyme disease. Thirty-six healthy adults from 18 to 65 years old, not previously exposed to Lyme disease, were randomly assigned to one of three vaccination groups. One group received two doses of placebo. The second group received two 0.5 milliliter (ml) doses of 10 micrograms of adjuvanted OspA one month apart. The third group received two 0.5 ml doses of 10 micrograms unadjuvanted OspA one month apart and a third dose six months from the beginning of the study. Participants were followed medically for one year. Three weeks after the first immunization, antibody immunoglobulin G levels increased approximately five-fold above levels found at the beginning of the study in both groups receiving active vaccine. After the second immunization, antibody levels increased approximately 40-fold among those receiving the active vaccines. Antibody levels eventually decreased to five-fold above those found at the beginning of the study. Side effects, including joint pain, were minor and resolved spontaneously.
AUTHOR ABSTRACT: Objective.--To evaluate the safety and immunogenicity of a recombinant outer surface lipoprotein A (OspA) Lyme vaccine in healthy adults. Design.--Randomized, double-blind, placebo-controlled trial. Setting.--Clinical research unit of a medical center. Participants.--Thirty-six healthy adult volunteers aged 18 through 65 years. Interventions.--Volunteers were randomly assigned to receive two 10-[mu]g doses of OspA Lyme vaccine, OspA Lyme vaccine adsorbed to alum, or a buffer placebo. Subjects in the OspA Lyme vaccine group received a third dose. Patients were assessed after each vaccination for a total follow-up period of 1 year. Serum samples for antibody determination were drawn at baseline, 2 and 3 weeks after dose 1, once per week for 4 weeks after dose 2,20 weeks after dose 2, and 1 month after dose 3. Main Outcome Measures.--Local and systemic adverse reactions and antibody levels specific for OspA. Results.--The most common reactions were local pain and tenderness at the injection site. Adverse events did not increase following the second or third dose. Two doses of both vaccine formulations elicited high-titer antibodies that inhibited replication of Borrelia burgdorferi in vitro. No differences were noted in antibody levels elicited by the adsorbed and nonadsorbed formulations. Conclusion.--Two or three doses of OspA Lyme vaccine are safe and immunogenic in adults. JAMA. 1994;271:1764-1768)

9/AB/48 (Item 4 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01481418 SUPPLIER NUMBER: 15285657 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Lyme borreliosis: basic science and clinical aspects.
Pfister, Hans-Walter; Wilske, Bettina; Weber, Klaus
The Lancet, v343, n8904, p1013(4)
April 23,
1994
PUBLICATION FORMAT: Magazine/Journal ISSN: 0099-5355 LANGUAGE: English
RECORD TYPE: Fulltext; Abstract TARGET AUDIENCE: Professional
WORD COUNT: 3529 LINE COUNT: 00310

ABSTRACT: Lyme disease is caused by infection with Borrelia burgdorferi and may occur in three stages. Lyme disease is transmitted by a tick bite. However, the bite may be unnoticed because the ticks that carry the disease

are small. A rash known as erythema migrans develops during the first stage of infection. The rash may appear from three days to 16 weeks after infection. It usually disappears within a few weeks or months. During the second stage of infection several organs may be affected. The patient may develop meningitis, cranial neuritis or heart problems. Joint pain may also develop. In the third stage, chronic organ problems may occur. The skin may atrophy. Inflammation and degeneration of the nerves may also occur. Blood tests commonly used to diagnose Lyme disease include the enzyme-linked immunosorbent assay, indirect immunofluorescence assay and Western blot. However, less than half of the patients with stage one disease have detectable antibodies to the organism.

9/AB/49 (Item 5 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01435697 SUPPLIER NUMBER: 14417486 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Molecular epidemiology and its clinical application. (Editorial)
Lupski, James R.
JAMA, The Journal of the American Medical Association, v270, n11, p1363(2)
Sept 15,
1993
DOCUMENT TYPE: Editorial PUBLICATION FORMAT: Magazine/Journal ISSN: 0098-7484 LANGUAGE: English RECORD TYPE: Fulltext; Abstract
TARGET AUDIENCE: Professional
WORD COUNT: 1862 LINE COUNT: 00163

ABSTRACT: Molecular biology techniques are being used more frequently in epidemiological investigations. These investigations examine the cause of disease outbreaks. Molecular biology techniques involve the genetic analysis of disease-causing microorganisms. Researchers used these techniques to investigate disease outbreaks caused by drug-resistant Staphylococcus aureus (S. aureus). They investigated the cause of recurrent epidemics in a well-baby nursery caused by erythromycin-resistant S. aureus. They found that one strain of bacteria was causing the epidemics. In another study, researchers investigated outbreaks of infection caused by methicillin-resistant S. aureus at a university hospital. Different strains of bacteria were responsible for the infection outbreaks. Certain criteria need to be satisfied before molecular methods can be used routinely in epidemiology. These criteria also need to be met before they gain widespread acceptance.

9/AB/50 (Item 6 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01423224 SUPPLIER NUMBER: 14018218 (USE FORMAT 7 OR 9 FOR FULL TEXT)
The biological and social phenomenon of Lyme disease.
Barbour, Alan G.; Fish, Durland
Science, v260, n5114, p1610(7)
June 11,
1993
PUBLICATION FORMAT: Magazine/Journal ISSN: 0036-8075 LANGUAGE: English
RECORD TYPE: Fulltext; Abstract TARGET AUDIENCE: Academic
WORD COUNT: 8588 LINE COUNT: 00665

AUTHOR ABSTRACT: Lyme disease, unknown in the United States two decades ago, is now the most common arthropod-borne disease in the country and has caused considerable morbidity in several suburban and rural areas. The

emergence of this disease is in part the consequence of the reforestation of the northeastern United States and the rise in deer populations. Unfortunately, an accurate estimation of its importance to human and animal health has not been made because of difficulties in diagnosis and inadequate surveillance activities. Strategies for prevention of Lyme disease include vector control and vaccines.

9/AB/51 (Item 7 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01358184 SUPPLIER NUMBER: 12177052 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Invasion of the central nervous system by Borrelia burgdorferi in acute disseminated infection.
Luft, Benjamin J.; Steinman, Charles R.; Neimark, Harold C.; Muralidhar, Bethi; Rush, Thomas; Finkel, Michael F.; Kunkel, Mark; Dattwyler, Raymond J.
JAMA, The Journal of the American Medical Association, v267, n10, p1364(4)
March 11,
1992
PUBLICATION FORMAT: Magazine/Journal ISSN: 0098-7484 LANGUAGE: English
RECORD TYPE: Fulltext; Abstract TARGET AUDIENCE: Professional
WORD COUNT: 3647 LINE COUNT: 00304

ABSTRACT: Involvement of the central nervous system (CNS) may occur early in the course of infection in patients with Lyme disease. Lyme disease is an infection characterized by fever, headache and fatigue caused by the bacterium Borrelia burgdorferi. Samples of cerebrospinal fluid (CSF) from 12 patients with Lyme disease and 16 healthy individuals were tested for the presence of the bacterium. The CSF of eight of the patients with Lyme disease was infected by Borrelia burgdorferi compared to none of the healthy individuals. Of the eight patients with Borrelia burgdorferi in their CSF, only four had any symptoms of CNS infection. Four of the patients with CNS infection had skin lesions, and the other four had acute cranial neuritis, or inflammation of cranial nerves. Patients with Lyme disease should be given antibiotics that can treat infections in the CSF.

9/AB/52 (Item 8 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01252786 SUPPLIER NUMBER: 09237360 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Positive Lyme serology in subacute bacterial endocarditis: a study of four patients.
Kaell, Alan T.; Volkman, David J.; Gorevic, Peter D.; Dattwyler, Raymond J.
JAMA, The Journal of the American Medical Association, v264, n22, p2916(3)
Dec 12,
1990
PUBLICATION FORMAT: Magazine/Journal ISSN: 0098-7484 LANGUAGE: English
RECORD TYPE: Fulltext; Abstract TARGET AUDIENCE: Professional
WORD COUNT: 1737 LINE COUNT: 00229

ABSTRACT: Lyme disease, or borreliosis, is caused by the tick-borne bacteria Borrelia burgdorferi, which causes inflammation of one or more systems, including skin, joints, nervous system, and heart. A classic rash occurs in 70 percent of patients, but many symptoms are nonspecific and reminiscent of other systemic disorders. Detection of Lyme disease is often accomplished by measuring blood levels of antibodies formed against the bacteria. Four cases are described of patients who were diagnosed with

Lyme disease on the basis of blood antibody levels. However, further testing indicated that they had bacterial endocarditis, an infection of the membrane lining the heart. The patients lived in areas with a high prevalence of Lyme disease, and one had developed the classic rash. Major symptoms included musculoskeletal complaints, fever, and malaise. Antibiotic treatment for Lyme disease provided temporary relief, but did not relieve the underlying endocarditis. Although intensive analysis of blood antibodies was suggestive of past infection with borrelia in two patients, positive results from the two remaining patients may have resulted from cross-reactivity of standard tests with other bacteria. The authors conclude that positive blood tests for Lyme disease may indicate previous exposure to the bacteria and past Lyme disease, but not active infection. In addition to laboratory testing, patients suspected of having Lyme disease require thorough clinical evaluation. Tests that actually identify B. burgdorferi, its DNA or its antigens are needed. (Consumer Summary produced by Reliance Medical Information, Inc.)

9/AB/53 (Item 9 from file: 149)
DIALOG(R)File 149:TGG Health&Wellness DB(SM)

01197119 SUPPLIER NUMBER: 08265629 (USE FORMAT 7 OR 9 FOR FULL TEXT)
Association of IDDM and attenuated response of 2',5'-oligoadenylate synthetase to yellow fever vaccine.
Bonnevie-Nielsen, V.; Larsen, M.L.; Frifelt, J.J.; Michelsen, B.; Lernmark, A.
Diabetes, v38, n12, p1636(7)
Dec,
1989
PUBLICATION FORMAT: Magazine/Journal ISSN: 0012-1797 LANGUAGE: English
RECORD TYPE: Fulltext; Abstract TARGET AUDIENCE: Professional
WORD COUNT: 4254 LINE COUNT: 00367

ABSTRACT: The body's natural defense system, the immune system, consists of cells and factors that inactivate and destroy invading foreign particles, such as viruses. When cells are exposed to viruses, they produce proteins called interferons (IFN), which are important in the immune reaction. IFN increases the production of tissue antigens, which activate the immune system to produce cells and antibodies against these antigens, and this initiates the elimination of the virus. The activity of the enzyme 2',5'-oligoadenylate synthetase (2',5'A) in white blood cells increases when the immune system and interferon are stimulated, and thus can be used as a measure of their activation. The activity of 2',5'A under basal or unstimulated conditions, and under stimulated conditions, specifically in the presence of yellow fever vaccine, was examined in the white blood cells of insulin-dependent diabetic patients. Basal enzyme activity was higher and stimulated enzyme activity was lower in diabetic patients than in normal subjects. Enzyme activity was related to whether the patient had previous viral infections. The higher basal 2',5'A activity in diabetic patients as compared to the controls may result from previous exposure to viruses, which would activate IFN and the immune system. The white blood cells from diabetic patients also had increased sensitivity to interferon. These results show that 2',5'A activity provides a measure of interferon activity and previous viral infections, resulting in activation of the immune system.

9/AB/54 (Item 1 from file: 162)
DIALOG(R)File 162:CAB HEALTH

00348754 CAB Accession Number: 890595691
Enzyme-linked immunosorbent assays for Lyme disease: reactivity of subunits of Borrelia burgdorferi.
Magnarelli, L. A.; Anderson, J. F.; Barbour, A. G.
Dep. Entomology, Connecticut Agric. Exp. Sta., New Haven, CT, USA.
Journal of Infectious Diseases vol. 159 (1): p.43-49
Publication Year: 1989
ISSN: 0022-1899
Language: English
Document Type: Journal article
The authors) prepared fractions of Borrelia burgdorferi , the etiologic agent of Lyme disease, from cultured spirochetes and used them as antigen in an enzyme-linked immunosorbent assay (ELISA) for IgG antibody. Polystyrene plates coated with an extract containing major proteins with apparent molecular masses of 34, 39, 59, and 68 kilodaltons had comparable sensitivity but greater specificity than plates coated with whole cells. Of the 33 serum specimens from individuals with Lyme disease that reacted with whole cells of B. burgdorferi in the class-specific ELISA, 30 (91%) remained positive when this extract was used. Cross-reactivity was minimal with antibody to treponemes. Use of subunit antigens may improve serological diagnosis of Lyme disease."Use of extracts containing predominantly flagella antigen (Kd41) gave only 17 out of 33 positive results in the authors' test. The use of the antigen with the more specific OspA gave positive results only late (and sometimes never) in the late disease. More work needs to be done in determining the best ' diagnostic ' antigen.D.J.M. Wright. 35 ref.

9/AB/55 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE

07101316 EMBASE No: 1997383180
Isolation and electron microscope examination of Bulgarian borrelia burgdorferi strains
Christova I.; Tzvetanov J.
Dr. I. Christova, NCIPD, Dept. of Microbiology, 26 Yanko Sakazov blvd., 1504 Sofia Bulgaria
Problems of Infectious and Parasitic Diseases (PROBL. INFECT. PARASIT. DIS.) (Bulgaria) 1997, 24/2 (30-31)
CODEN: PIPDD ISSN: 0204-9155
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 8

Cultivation of B. burgdorferi from ixodes ricinus ticks and clinical specimens continue to be laborious and not effective procedures. One hundred thirty one unfed I.ricinus ticks were harvested by flagging from vegetation. Skin biopsy specimens were taken from 12 patients with erythema migrans. Thirteen B. burgdorferi strains were isolated from tick cultures and 3 from 12 skin biopsies. The earliest detected borrelial grouth was 6 days after inoculating medium, the latest - 26 days after inoculation. Two of the tick and two of the skin isolates were examined by electron microscope. The morphology of the cells was similar if not identical and differed from the described one for American and Swedish isolates. Cells of the laboratory isolates had 8 flagella , surface layer possessed regular substructure delineated by a single line and the ends of the cells were blunt. These are the largest series of isolated B. burgdorferi strains from ticks in Bulgaria. The report is the first for isolation of B. burgdorferi strains from skin biopsies in Bulgaria and for examination the

ultrastructure of local B. burgdorferi strains.

9/AB/56 (Item 1 from file: 185)
DIALOG(R)File 185:Zoological Record Online(R)

00106136 BIOSIS No. 11500006136 Long Record
The Dermaptera of Africa. Part 2.
Brindle A
JOURNAL: KONINKLIJK MUSEUM VOOR MIDDEN-AFRIKA TERVUREN BELGIE ANNALEN ZOOLOGISCHE WETENSCHAPPEN No. 225 1978: 1-204, illustr.
DOCUMENT TYPE: Article
ISSN: 0379-1785

9/AB/57 (Item 1 from file: 351)
DIALOG(R)File 351:Derwent WPI

009141222
WPI Acc No: 1992-268660/ 199232
Related WPI Acc No: 1993-288400
XRAM Acc No: C92-119870
New flagella-less Borrelia and derived antigens - useful for vaccinating against and diagnosing Borrelia infections e.g. Lyme disease or relapsing fever
Patent Assignee: UNIV TEXAS (TEXA); UNIV TEXAS SYSTEM (TEXA)
Inventor: BARBOUR A G; BUNDOC V; BUNDOC V G; SADZIENE A
Number of Countries: 037 Number of Patents: 006
Patent Family:

Patent No	Kind	Date	Applicat No	Kind	Date	Week	
WO 9212235	A1	19920723	WO 92US181	A	19920110	199232	B
AU 9212269	A	19920817	AU 9212269	A	19920110	199245	
			WO 92US181	A	19920110		
EP 575348	A1	19931229	EP 92903976	A	19920110	199401	
			WO 92US181	A	19920110		
US 5436000	A	19950725	US 91641143	A	19910111	199535	
US 5585102	A	19961217	US 91641143	A	19910111	199705	
			US 93124290	A	19930920		
US 6077515	A	20000620	US 91641143	A	19910111	200035	
			US 93124290	A	19930920		
			US 96696372	A	19960813		

Priority Applications (No Type Date): US 91641143 A 19910111; US 93124290 A 19930920; US 96696372 A 19960813
Patent Details:

Patent No	Kind	Lan	Pg	Main IPC	Filing Notes
WO 9212235	A1	E	88	C12N-001/20	
Designated States (National): AT AU BB BG BR CA CH CS DE DK ES FI GB HU JP KP KR LK LU MG MN MW NL NO PL RO RU SD SE					
Designated States (Regional): AT BE CH DE DK ES FR GB GR IT LU MC NL OA SE					
AU 9212269	A			C12N-001/20	Based on patent WO 9212235
EP 575348	A1	E		C12N-001/20	Based on patent WO 9212235
Designated States (Regional): AT BE CH DE DK ES FR GB GR IT LI LU MC NL SE					
US 5436000	A		33	A61K-039/02	
US 5585102	A		27	C12N-001/00	Cont of application US 91641143
US 6077515	A			A61K-039/00	Cont of application US 91641143
					Cont of application US 93124290

Cont of patent US 5436000
Cont of patent US 5585102

Abstract (Basic): WO 9212235 A

A culture, or compsn. of matter, of a selected species of a flagellaless Borrelia microorganism (I) is new. Also new are: (a) a compsn. of matter comprising a purified prepn. of antigens derived from a culture of (I); (b) an immunoassay for the detection of a Borrelia infection comprising: contacting a body fluid sample with (a) under conditions that favour binding of antibodies (Abs) reactive with the antigens; and detecting the binding; (c) a kit for immunoassay comprising a compartmentalised carrier, and a first container containing (a); and (d) a vaccine for Borreliosis or Lyme disease comprising (a) and a pharmaceutical carrier.

USE/ADVANTAGE - (I), vaccines and immunoassays may be used for the following borreliae infections: Lyme disease; epidemic bovine abortion; avian spirochetosis; or relapsing fever.f a

Dwg.0/7

Abstract (Equivalent): US 5585102 A

A kit for immunoassay comprising:

(a) a carrier compartmentalized to contain one or more containers; and

(b) a first container containing an antigenic preparation comprising whole cells of a flagella -less strain of Borrelia burgdorferi .

Dwg.0/7

US 5436000 A

Pure culture of flagella -less B. burgdorfen has the ATCC Accession No. 55131.

USE/ADVANTAGE - As line vaccines against and for the diagnosis of lyme disease. Also for preventing relapsing fever and avian spirocheltosis.Suitable for humans, dogs, horses, equids, cattle, deer and rodents also birds esp. poultry. Admin. is 0.01 mcg.-1 mg. pref. 1-100 mcg. Admin. is by subcutaneous or intramuscular injection. Safer than whole cell B.burgdorfen vaccines, and does not form vaccine-induced auto-antibodies associated with whole cell vaccines or contaminated subunit vaccines.

Dwg.0/14

?

Set	Items	Description
S1	21	LYME(5N)ASSAY?

1/3,AB,CM/1 (Item 1 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02638662

Utility
METHODS FOR DIAGNOSING EARLY LYME DISEASE

PATENT NO.: 5,620,862
ISSUED: April 15, 1997 (19970415)
INVENTOR(s): Padula, Steven J., Simsbury, CT (Connecticut), US (United States of America)
ASSIGNEE(s): University of Connecticut, (A U.S. Company or Corporation), Storrs, CT (Connecticut), US (United States of America)
[Assignee Code(s):
APPL. NO.: 8-158,353
FILED: November 24, 1993 (19931124)

GOVERNMENT SUPPORT

Work described herein was supported in whole or in part by a grant from Public Health Service (Grant No. #5R29-AR39361). The Government has certain rights in this invention.

FULL TEXT: 1310 lines

ABSTRACT

The invention relates to DNA encoding Borrelia burgdorferi sensu stricto outer surface protein C. Purified and recombinant forms of a 23 kDa protein from a Connecticut isolate of B. burgdorferi are described. The 23 kDa protein, referred to as p23 or OspC, can be used for immunodiagnostic **assays** for detection of early **Lyme** disease. The protein, amino acid coding for the protein and DNA sequences can be used to prevent Lyme disease, to diagnose/detect B. burgdorferi in human or animal tissues or body fluids. Antibodies specific for the protein can also be generated.

I claim:

1. A method for the detection of early stage of Lyme disease, comprising the steps:
a) contacting recombinant or substantially pure form of outer surface protein C (OspC) from Borrelia burgdorferi sensu stricto, wherein said recombinant form of OspC has been produced by host cells which express OspC encoded by heterologous DNA, to a biological sample from a mammal suspected of having Lyme disease, wherein said sample is taken from the mammal at the early stage of infection; and
b) detecting the presence or absence ok a complex formed between OspC and IgM antibodies, wherein the presence of an OspC/IgM complex is indicative of exposure to and infection by Borrelia burgdorferi.

2. The method of claim 1 wherein the biological sample is selected from the group consisting of serum, urine, tissue, cerebrospinal fluid, blood, pericardial fluid and synovial fluid.

3. A kit comprising recombinant or substantially pure form of outer surface protein C (OspC) from Borrelia burgdorferi sensu stricto, wherein said recombinant form of OspC has been produced by host cells, which express OspC encoded by heterologous DNA, and anti-human IgM conjugated to a detectable label for use in detecting the presence of IgM antibodies to the protein in a biological sample.

4. The kit of claim 3 further comprising Borrelia burgdorferi outer surface protein A, Borrelia burgdorferi outer surface protein B, Borrelia burgdorferi p41 and combinations thereof.

1/3,AB,CM/2 (Item 2 from file: 654)

02638645

Utility
IMMUNOASSAY DIAGNOSTIC KIT

PATENT NO.: 5,620,845
ISSUED: April 15, 1997 (19970415)
INVENTOR(s): Gould, Martin, Gibbstown, NJ (New Jersey), US (United States of America)
Vulimiri, Sudhakar, West Deptford, NJ (New Jersey), US (United States of America)
ASSIGNEE(s): Ampcor, Inc, (A U.S. Company or Corporation), Bridgeport, NJ (New Jersey), US (United States of America)
[Assignee Code(s):
APPL. NO.: 8-306,250
FILED: September 14, 1994 (19940914)

REFERENCE TO RELATED APPLICATION

This application is a continuation of application Ser. No. 07-917,916, filed Jul. 21, 1992 and now abandoned, which is a continuation of Ser. No. 07-447,594, filed Dec. 8,1989 and now abandoned, which is a continuation-in-part of Ser. No. 07-361,878, filed Jun. 6, 1988 and now abandoned.

FULL TEXT: 590 lines

ABSTRACT

An immunoassay process is provided for the detection of a target antigen in a fluid sample where an admixture of the target antigen and a labeled capture reagent against the target antigen is contacted by a solid polymeric carrier having bound to a portion of its surface a capture reagent against the target antigen and visual determination of a change in color of the bound labeled reaction product on the surface of the solid carrier member serves to readily detect the presence of the target antigen.

What is claimed is:

1. An immunoassay process for the detection of a target immunologically active agent in a liquid sample comprising:
a) contacting said liquid sample containing said target immunologically active agent to be assayed with a labeled capture reagent against said target immunologically active agent, and with a controlled effective amount of a bound capture reagent against said target immunologically active agent bound to a solid carrier member over only a portion thereof in a controlled substantially specific array and wherein the remaining portion of said solid carrier member having been treated with animal serum and with a solution of a casein protein in an effective amount is substantially blocked against bonding to said labeled capture reagent and said target immunologically active agent; and
b) detecting the presence of said target immunologically active agent by determining the label bound to said bound capture reagent on said solid carrier member as an indication of the presence of the target immunologically active agent in said fluid sample.

2. The immunoassay process according to claim 1, wherein the presence of target immunologically active agent is detected without washing the solid carrier member.

3. The process according to claim 2, wherein said target immunologically active agent is an antigen.

4. The process according to claim 3, wherein said labeled capture reagent

is an enzyme labeled antibody against said target antigen.

5. The immunoassay process according to claim 3, wherein said solid carrier member is a film of non-fibrous material and said bound capture reagent is applied to and bound over only a portion of one surface of said solid carrier member by jet-type atomizer means in a controlled narrow linear band containing a controlled amount of said bound capture reagent.

6. The immunoassay process according to claim 5, wherein said solid carrier member is a polymeric material to which said bound capture reagent is bound.

7. The immunoassay process according to claim 6, wherein said bound capture reagent consists essentially of an immobilized antibody against said target immunologically active agent and said labeled capture reagent is an antibody reagent against said target immunologically active agent to which is attached a label.

8. The process according to claim 2, wherein said labeled capture reagent and said bound capture reagent are monoclonal or polyclonal antibodies or mixtures thereof.

9. The process according to claim 2, wherein said liquid sample is a body fluid, culture media, food or water.

10. The process according to claim 1, wherein said labeled capture reagent is an enzyme labeled antibody and the determining label step comprises contacting said solid carrier member with a color forming solution selected to generate a color change of the enzyme label which is visual.

11. The process according to claim 10, wherein said color forming solution is in a time-release form in an admixture of said fluid sample of target immunologically active agent and said labeled capture reagent.

12. An immunoassay process for the detection of a target immunologically active agent in a liquid sample consisting essentially of admixing said liquid sample containing said target immunologically active agent with a labeled capture reagent against said target immunologically reactive agent, contacting said admixture with a controlled effective amount of a bound capture reagent against said target immunologically active agent, said bound capture reagent being applied to and bound to a solid carrier member by jet-type atomizer means over only one surface thereof in a controlled substantially specific array and wherein the remaining portion of said solid carrier member is substantially blocked against bonding to said labeled capture reagent and said target immunologically reactive agent, and then contacting said bound capture reagent without washing the same with a color forming solution selected for detecting the presence of the target immunologically active agent by determining by visualization an indication of the presence of label bound to said bound capture reagent on said carrier member.

13. The immunoassay process according to claim 12, wherein said target immunologically active agent is an antigen.

14. The immunoassay process according to claim 13, wherein said solid carrier member is a polymeric material to which said bound capture reagent is bound and wherein the remaining portion of said solid carrier member is treated with animal serum and with a solution of a casein protein in an effective amount to substantially block the same.

15. The immunoassay process according to claim 14, wherein said bound capture reagent consists essentially of an immobilized antibody reagent against said target immunologically active agent and said labeled capture reagent is an antibody reagent against said target immunologically active agent to which is attached a label.

16. The immunoassay process according to claim 15, wherein said solid carrier member is a thin film of non-fibrous polymeric material to which

said bound capture reagent is bound in a controlled linear or dot-like pattern consisting essentially of a controlled effective amount of said bound capture reagent.

17. The immunoassay process according to claim 16, wherein said bound capture reagent is chemically and/or absorptively bound to said carrier member.

18. The immunoassay process according to claim 16, wherein said labeled antibody reagent is an enzyme labeled antibody reagent.

19. The immunoassay process according to claim 13, wherein said antigen is selected from the group consisting of chorigonadotropin, Salmonella, Epstein-Barr, Chlamydia, an antigen of Lyme disease, Escherichia coli, Proteus, Kiebsiella, Staphylococcus, Pseudomonas and Hepatitis A & B.

20. The immunoassay process according to claim 12, wherein said solid carrier member is removed from contact with said reaction admixture and is directly placed in contact with said color-forming solution in a separate container without washing.

02600991
Utility

FLAGELLA-LESS BORRELIA

PATENT NO.: 5,585,102
ISSUED: December 17, 1996 (19961217)
INVENTOR(s): Barbour, Alan G., San Antonio, TX (Texas), US (United States of America)
Bundoc, Virgilio G., Newbury Park, CA (California), US (United States of America)
Sadziene, Adriadna, San Antonio, TX (Texas), US (United States of America)
ASSIGNEE(s): Board of Regents, The University of Texas System, (A U.S. Company or Corporation), Austin, TX (Texas), US (United States of America)
[Assignee Code(s): 83960]
EXTRA INFO: Assignment transaction [Reassigned], recorded December 21, 1996 (19961221)
APPL. NO.: 8-124,290
FILED: September 20, 1993 (19930920)
This application is a continuation of application Ser. No. 07-641,143, filed Jan. 11, 1993, now U.S. Pat. No. 5,436,000.

FUNDING

Development of the present invention was aided in part by finding from The National Institute of Health, grant no. AI24424. Accordingly, the U.S. Government has a paid-up license and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided by the terms of Grant No. AI24424.

ABSTRACT

This invention relates to flagella-less strains of Borrelia to novel methods for use of the microorganisms as vaccines and in diagnostic assays. Although a preferred embodiment of the invention is directed to Borrelia burgdorferi, the present invention encompasses flagella-less strains of

other microorganisms belonging to the genus Borrelia. Accordingly, with the aid of the disclosure, flagella-less mutants of other Borrelia species, e.g., B. coriacei, which causes epidemic bovine abortion, B. anserina, which causes avian spirochetosis, and B. recurrentis and other Borrelia species causative of relapsing fever, such as Borrelia hermsii, Borrelia turicatae, Borrelia duttoni, Borrelia persica, and Borrelia hispanica, can be prepared and used in accordance with the present invention and are within the scope of the invention. Therefore, a preferred embodiment comprises a composition of matter comprising a substantially pure preparation of a strain of a flagella-less microorganism belonging to the genus Borrelia.

We claim:

1. A kit for immuneassay comprising:
(a) a carrier compartmentalized to contain one or more containers; and
(b) a first container containing an antigenic preparation comprising whole cells of a flagella-less strain of Borrelia burgdorferi.

2. The kit of claim 1 wherein said antigenic preparation is immobilized on a solid matrix.
3. The kit of claim 1 further comprising a second container comprising a preparation of antibodies reactive with antigens in the antigenic preparation.

4. The kit of claim 3 further comprising a third container containing a detection reagent.

5. A kit for immunoassay comprising:
(a) carrier compartmentalized to contain one or more containers; and
(b) a first container containing an antigenic preparation comprising whole cells of Borrelia burgdorferi wherein said Borrelia is the strain of B. burgdorferi deposited with the American Type Culture Collection having Accession No. 55131.

6. An antigenic preparation comprising whole cells of flagella-less Borrelia burgdorferi microorganisms.

1/3,AB,CM/4 (Item 4 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02598669
Utility
DNA ENCODING BORRELIA BURGDORFERI OSPA AND A METHOD FOR DIAGNOSING BORRELIA BURGDORFERI INFECTION

PATENT NO.: 5,582,990
ISSUED: December 10, 1996 (19961210)
INVENTOR(s): Bergstrom, Sven, Umea, SE (Sweden)
Barbour, Alan G., San Antonio, TX (Texas), US (United States of America)
Magnarelli, Louis A., Durham, CT (Connecticut), US (United States of America)
ASSIGNEE(s): Symbicom Aktiebolag, (A Non-U.S. Company or Corporation), SE (Sweden)
[Assignee Code(s): 21342]
APPL. NO.: 8-320,416
FILED: October 03, 1994 (19941003)
PRIORITY: 5902-88, DK (Denmark), October 24, 1988 (19881024)

This is a division of copending parent application Ser. No. 08-079,601, filed Jun. 22, 1993 itself a continuation of Ser. No. 07-924,798, filed Aug. 6, 1992 now abandoned, which is also a continuation of Ser. No. 07-422,881, filed Oct. 18, 1989 now abandoned, all hereby incorporated by reference in their entirety.

FULL TEXT: 2416 lines

ABSTRACT

Disclosed and claimed are isolated nucleic acid molecules, such as DNA encoding Borrelia burgdorferi OspA, vectors containing the nucleic acid molecules, and methods for diagnosing Borrelia burgdorferi infection employing such nucleic acid molecules. The isolated nucleic acid molecule can be an isolated DNA molecule encoding the 31 kD OspA protein of New York strain B31. The isolated nucleic acid molecule also can be an isolated DNA molecule encoding Borrelia burgdorferi OspA and a signal peptide which contains an amino acid recognition sequence. The recognition sequence can be L-z-z-C, where each z independently designates a small, neutral amino acid, such as isoleucine or alanine. The recognition sequence can also be L-I-x-C where x is a non-charged amino acid residue, such as alanine. Further, the isolated nucleic acid molecule can be an isolated DNA molecule encoding Borrelia burgdorferi OspA and which includes a 5'-flanking region containing at least one promoter sequence for expression of the OspA. And additionally, the isolated nucleic acid molecule can be an isolated DNA molecule encoding Borrelia burgdorferi OspA as a fusion polypeptide containing the OspA.

We claim:

1. An isolated DNA molecule consisting of a nucleotide sequence encoding OspA protein of Borrelia burgdorferi as set forth in FIG. 5.

2. The isolated DNA molecule of claim 1 wherein the OspA protein is the 31 kd OspA protein of New York strain B31.

3. The isolated DNA molecule of claim 1 which has the nucleotide sequence set forth in FIG. 5.

4. The isolated DNA molecule of claim 1 including a nucleotide sequence encoding a signal peptide which contains an amino acid recognition sequence.

5. The isolated DNA molecule of claim 4 wherein the recognition sequence is L-z-z-C, wherein each z independently designates a small, neutral amino acid.

6. The isolated DNA molecule of claim 5 wherein each Z is independently selected from the group consisting of isoleucine and alanine.

7. The isolated DNA molecule of claim 4 wherein the recognition sequence is L-I-x-C, wherein x is a non-charged amino acid residue, and the recognition sequence is in the C-terminal region of the signal peptide.

8. The isolated DNA molecule of claim 7 wherein x is alanine.

9. The isolated DNA molecule of claim 1 including a 5'-flanking region containing at least one promoter sequence for expression of the OspA protein.

10. The isolated DNA molecule of claim 1 wherein the nucleotide sequence encodes a fusion polypeptide containing the OspA protein.

11. A vector containing the isolated DNA molecule of claim 1.

12. A method for detecting Borrelia burgdorferi in a host by detecting the presence of Borrelia burgdorferi DNA coding for OspA, which method comprises:
obtaining a DNA sample from the host,
contacting the DNA with DNA specific to Borrelia burgdorferi under amplification conditions, whereby amplification occurs only if Borrelia burgdorferi DNA is present in the DNA sample, and

detecting the presence of amplified Borrelia burgdorferi DNA and thereby detecting the presence of Borrelia burgdorferi in the host, whereby the DNA specific to Borrelia burgdorferi is the DNA molecule set forth in claim 1.

1/3,AB,CM/5 (Item 5 from file: 654)

02598515

Utility
SONICATED BORRELIA BURGDORFERI VACCINE

PATENT NO.: 5,582,829
ISSUED: December 10, 1996 (19961210)
INVENTOR(s): Alliger, Howard M., Melville, NY (New York), US (United States of America)
Frey, Alan, Highland Park, NJ (New Jersey), US (United States of America)
ASSIGNEE(s): Rx Technologies, Inc, (A U.S. Company or Corporation), Garden City, NY (New York), US (United States of America)
[Assignee Code(s):
APPL. NO.: 7-921,303
FILED: July 28, 1992 (19920728)

This application is a continuation-in-part application of U.S. patent application Ser. No. 07-505,193, filed Apr. 5, 1990, abandoned, entitled "Production of Vaccine from Viruses or Cells, Product Produced Thereby and Method of Use."

This work has been supported by DARPA Grant No. N0014-90-J-2032. The United States Government retains certain rights in the invention.

FULL TEXT: 1061 lines

ABSTRACT

A process for the preparation of a vaccine from substantially viable spirochetal bacteria of Borrelia, preferably Borrelia burgdorferi having immunogenic or therapeutic properties and capable of inducing an immune or therapeutic response against Lyme Disease when administered to a patient is described. The product for use against Lyme Disease is produced by ultrasound treatment of substantially viable spirochetal bacteria of Borrelia burgdorferi. The invention produces a product and a method of treatment that can be used for the immunization and/or therapy of a patient against Lyme Disease to minimize or prevent the contraction of the disease or to treat the disease.

We claim:

1. A product for administration into a patient as an immunizing or therapeutic constituent comprising a population of substantially viable spirochetal bacteria of at least two serovar of Borrelia burgdorferi disrupted by sonic energy without denaturing the protein components thereof in combination with an effective amount of an adjuvant.

2. A vaccine for administration into a patient as an immunizing or therapeutic constituent comprising immunogenic material prepared by disrupting a population of at least two serovar of substantially viable spirochetal bacteria of Borrelia burgdorferi with sonic energy without denaturing the protein components thereof, an effective amount of which is combined with a pharmaceutically acceptable carrier.

3. The vaccine of claim 2 wherein said immunogenic material is further subjected to low speed centrifugation to isolate a supernatant which is combined with said carrier.

4. The vaccine of claim 2 prepared under anaerobic conditions.

5. The vaccine of claim 2 prepared by disrupting said spirochetal bacteria in an aqueous medium which minimizes the formation of undesirable free radicals.

6. The vaccine according to claim 2 wherein said immunogenic material is combined with an effective amount of an adjuvant in said carrier.

7. A method for vaccinating a patient against Lyme Disease comprising the step of administering to said patient a vaccine comprising an effective amount of immunogenic material prepared by the sonication of substantially viable spirochetal bacteria of Borrelia burgdorferi disrupted by sonic energy without denaturing the protein components thereof.

8. The method according to claim 7 wherein said vaccine is administered as a sterile solution.

9. The method of claim 7 wherein said vaccine contains an effective amount of an adjuvant.

10. The method according to claim 7 wherein said vaccine includes a pharmaceutically acceptable carrier.

11. The method according to claim 9 wherein said immunogenic material is further subjected to low speed centrifugation to isolate a supernatant which is combined with said adjuvant.

12. The method according to claim 7 wherein said immunogenic material is obtained from more than one serovar of Borrelia burgdorferi.

13. A method of evoking an immunogenic or therapeutic response in a patient comprising the step of administering to the patient an effective amount of an immunogenic material prepared by disrupting a population of least two serovar of substantially viable spirochetal bacteria of Borrelia burgdorferi with sonic energy without denaturing the protein components thereof.

14. The method according to claim 13 wherein said immunogenic material is combined with a pharmaceutically acceptable carrier.

15. The method according to claim 13 wherein said disrupted immunogenic material is thereafter subjected to low speed centrifugation.

16. The method according to claim 13 wherein said immunogenic material is combined with an adjuvant.

1/3,AB,CM/6 (Item 6 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02567327
Utility
RECOMBINANT VACCINE AGAINST LYME DISEASE

PATENT NO.: 5,554,371
ISSUED: September 10, 1996 (19960910)
INVENTOR(s): Caputa, Anthony C., Nanuet, NY (New York), US (United States of America)
Bey, Russell F., Arden Hills, MN (Minnesota), US (United States of America)
Murtaugh, Michael P., Roseville, MN (Minnesota), US (United States of America)
ASSIGNEE(s): Regents of the University of Minnesota, (A U.S. Company or Corporation), Minneapolis, MN (Minnesota), US (United States of America)

[Assignee Code(s): 56024]
APPL. NO.: 8-227,478
FILED: April 14, 1994 (19940414)

This is a continuation of application Ser. No. 07-790,332, filed Nov. 12, 1991, now abandoned.

FULL TEXT: 825 lines

ABSTRACT

A highly-antigenic, recombinant polypeptide of a molecular weight of about 110-kD by SDS-PAGE is disclosed, which is derived by transforming E. coli with a 7.1 kB DNA fragment from EcoR1-digested B. burgdorferi DNA, followed by identification of cloned transformants expressing polypeptides which bind to anti-B. burgdorferi antibodies.

What is claimed is:

1. A vaccine comprising an immunogenic amount of an antigenic recombinant polypeptide of about 110-kD molecular weight, as determined under reducing conditions by SDS-PAGE, wherein said polypeptide is combined with a physiologically acceptable, non-toxic liquid vehicle, which amount is effective to immunize a susceptible mammal against Lyme borreliosis, wherein the amino acid sequence of said polypeptide corresponds to the amino acid sequence of the recombinant 110-kD polypeptide produced by E. coli transformant ATCC 68825.

2. The vaccine of claim 1 which further comprises an effective amount of an immunological adjuvant.

3. The vaccine of claim 1 wherein the mammal is a dog, a cat, a llama, a bovine, sheep, goat or a horse.

4. The vaccine of claim 1 wherein the mammal is a human.

5. A method of protecting a susceptible mammal against Lyme borreliosis comprising administering to said mammal an effective amount of a vaccine comprising any immunogenic amount of the recombinant protein of claim 1 in combination with a physiologically-acceptable non-toxic liquid vehicle, which amount is effective to immunize the susceptible mammal against Lyme borreliosis.

6. The method of claim 5 wherein the vaccine is administered by subcutaneous or intramuscular injection.

7. The method of claim 5 wherein the vaccine is administered by oral ingestion.

8. A recombinant polypeptide of about 110-kD molecular weight, as determined by SDS-PAGE under reducing conditions, wherein the amino acid sequence of said polypeptide corresponds to the amino acid sequence of the recombinant 110-kD polypeptide produced by E. coli transformant ATCC 68825.

1/3,AB,CM/7 (Item 7 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02533056
Utility
BORRELIA ANTIGEN

PATENT NO.: 5,523,089
ISSUED: June 04, 1996 (19960604)
INVENTOR(s): Bergstrom, Sven, Umea, SE (Sweden)

Barbour, Alan G., San Antonio, TX (Texas), US (United States of America)
Magnarelli, Louis A., Durham, CT (Connecticut), US (United States of America)
ASSIGNEE(s): Symbicom Aktiebolag, (A Non-U.S. Company or Corporation), SE (Sweden)
[Assignee Code(s): 21342]
APPL. NO.: 8-79,601
FILED: June 22, 1993 (19930622)
PRIORITY: 5902-88, DK (Denmark), October 24, 1988 (19881024)

This is a continuation of application Ser. No. 07-924,798 filed Aug. 6, 1992, which in turn was a continuation of application Ser. No. 07-422,881 filed Oct. 18, 1989, both now abandoned, the contents of all of which are hereby incorporated by reference.

FULL TEXT: 2510 lines

ABSTRACT

B fraction of Borrelia burgdorferi, methods for preparing the B fraction, and compositions containing the B fraction, are disclosed and claimed.

We claim:

1. B fraction of Borrelia burgdorferi obtained by the following steps comprising:
a) lysing B. burgdorferi spirochaete cells with a detergent so as to release outer membranae components from the cells but not denature said outer membrane components,
b) subsequently subjecting the lysed cells to centrifugation resulting in a first pellet comprising cell wall and flagellar components and a first supernatant comprising outer membrane components,
c) incubating the first supernatant from step b) under conditions sufficient to precipitate proteins of fraction E of the first supernatant followed by centrifugation so as to obtain a second pellet comprising fraction E and a second supernatant,
d) subjecting the second supernatant from step c) to filtration and dialyzing the supernatant against an aqueous medium with a low ionic strength so as to substantially remove the detergent and allow B. burgdorferi derived cell components to precipitate in the dialysis bag,
e) centrifuging the contents of the dialysis bag so as to obtain a third pellet comprising fraction B and a third supernatant comprising fraction C,
the fraction B containing lipids and being
substantially free from cell wall and flagellar components of B. burgdorferi,
substantially free from detergent, and
substantially free from sodium dodecyl sulphate.

2. A composition which binds with antibodies elicited by B. burgdorferi, said composition comprising an effective amount of fraction B for antibody binding, and a carrier or vehicle, wherein said fraction B is obtained by the following method comprising the steps:
a) lysing B. burgdorferi spirochaete cells with a detergent so as to release outer membranae components from the cells but not denature said outer membrane components,
b) subsequently subjecting the lysed cells to centrifugation resulting in a first pellet comprising cell wall and flagellar components and a first supernatant comprising outer membrane components,
c) incubating the first supernatant from step b) under conditions sufficient to precipitate proteins of fraction E of the first supernatant followed by centrifugation so as to obtain a second pellet comprising fraction E and a second supernatant,
d) subjecting the second supernatant from step c) to filtration and dialyzing the supernatant against an aqueous medium with a low ionic strength so as to substantially remove the detergent and allow B.

burgdorferi derived cell components to precipitate in the dialysis bag,
e) centrifuging the contents of the dialysis bag so as to obtain a third pellet comprising fraction B and a third supernatant comprising fraction C,
the fraction B containing lipids and being
substantially free from cell wall and flagellar components of B. burgdorferi,
substantially free from detergent, and
substantially free from sodium dodecyl sulphate.

3. The B fraction according to claim 1 which reacts by antibody binding with at least about 85% of sera from patients with Lyme disease, but with not more than about 20% of sera of syphilitic patients.

4. An immunogenic composition comprising an immunologically effective amount of B fraction as claimed in claim 1.

5. A diagnostic composition for the detection of B. burgdorferi antibodies in a sample, which comprises fraction B of B. burgdorferi wherein said fraction B is obtained by the following method comprising the steps:
a) lysing B. burgdorferi spirochaete cells with a detergent so as to release outer membranae components from the cells but not denature said outer membrane components,
b) subsequently subjecting the lysed cells to centrifugation resulting in a first pellet comprising cell wall and flagellar components and a first supernatant comprising outer membrane components,
c) incubating the first supernatant from step b) under conditions sufficient to precipitate proteins of fraction E of the first supernatant followed by centrifugation so as to obtain a second pellet comprising fraction E and a second supernatant,
d) subjecting the second supernatant from step c) to filtration and dialyzing the supernatant against an aqueous medium with a low ionic strength so as to substantially remove the detergent and allow B. burgdorferi derived cell components to precipitate in the dialysis bag,
e) centrifuging the contents of the dialysis bag so as to obtain a third pellet comprising fraction B and a third supernatant comprising fraction C,
the fraction B containing lipids and being
substantially free from cell wall and flagellar components of B. burgdorferi,
substantially free from detergent, and
substantially free from sodium dodecyl sulphate.

6. A method of preparing fraction B of B. burgdorferi which fraction B contains lipids, is substantially free from cell wall and flagellar components of B burgdorferi, is substantially free from detergent, and, is substantially free from sodium dodecyl sulphate, said method consisting essentially of the following sequential steps:
a) lysing B. burgdorferi spirochaete cells with a detergent so as to release outer membrane components from the cells but not denature said outer membrane components,
b) subsequently subjecting the lysed cells to centrifugation resulting in a first pellet comprising cell wall and flagellar components and a first supernatant comprising outer membrane components,
c) separating said first supernatant from said first pellet,
d) subsequently incubating the separated first supernatant from step c.) under conditions sufficient to precipitate proteins of fraction E of the first supernatant followed by centrifugation so as to obtain a second pellet comprising fraction E and a second supernatant,
e) separating said second supernatant from said second pellet,
f) subsequently subjecting the separated second supernatant from step e) to filtration to remove insoluble particles from said separated second supernatant,
g) subsequently dialyzing the filtered second supernatant against an aqueous medium with a low ionic strength so as to substantially remove the detergent and allow B. burgdorferi derived cell components to precipitate in the dialysis bag,
h) subsequently centrifuging the contents of the dialysis bag so as to obtain a third pellet comprising fraction B and a third supernatant

comprising fraction C, and
i) subsequently separating said third pellet from said third supernatant.

7. A method of preparing fraction B of B. burgdorferi which fraction B contains lipids, is substantially free from cell wall and flagellar components of B. burgdorferi, is substantially free from detergent, and, is substantially free from sodium dodecyl sulphate, said method, consisting essentially of the following sequential steps:
a) lysing B. burgdorferi spirochaete cells with a detergent so as to release outer membrane components from the cells but not denature said outer membrane components,
b) subsequently subjecting the lysed cells to centrifugation resulting in a first pellet comprising cell wall and flagellar components and a first supernatant comprising outer membrane components,
c) separating said first supernatant from said first pellet,
d) subsequently incubating the separated first supernatant from step c) under conditions sufficient to precipitate proteins of fraction E of the first supernatant followed by centrifugation so as to obtain a second pellet comprising fraction E and a second supernatant,
e) separating said second supernatant from said second pellet,
f) subsequently subjecting the separated second supernatant from step e) to filtration to remove insoluble particles from said separated second supernatant,
g) subsequently subjecting the filtered second supernatant to ultrafiltration so as to substantially remove the detergent and allow B burgdorferi derived cell components to precipitate in the retentate resulting from the ultrafiltration,
h) subsequently centrifuging the retentate so as to obtain a third pellet comprising fraction B and a third supernatant comprising fraction C, and
i) subsequently separating said third pellet from said third supernatant.

8. The method according to claim 6, wherein the detergent used in step a) is a water-dialyzable detergent selected from the group consisting of a non-ionic, and a zwitterionic.

9. The method according to claim 8, wherein the detergent is octyl- beta -D-glucopyranoside (OGP).

10. The method according to claim 6, wherein lysing step a) is performed at a temperature of about 20 degree(s) -60 degree(s) C., with the detergent having a concentration of about 0.1-2%, and incubating step d) is carried out at a temperature of about 45 degree(s) -65 degree(s) C.

11. The method according to claim 10, wherein lysing step a) is performed at a temperature of about 25 degree(s) -50 degree(s) C., with the detergent having a concentration of about 0.2-1% and incubating step d) is carried out at a temperature of about 50 degree(s) -60 degree(s) C.

12. The method according to claim 11, wherein lysing step a) is performed at a temperature of about 30 degree(s) -40 degree(s) C. and incubating step c) is carried out at a temperature of about 56 degree(s) C.

13. The method according to claim 12, wherein lysing step a) is performed at a temperature of about 37 degree(s) C.

14. The method according to claim 6, wherein the filtration to which the second supernatant obtained in step d) is subjected to in step e) is micro filtration through a membrane with a pore diameter of up to about 2.0 mu m.

15. The method according to claim 14, wherein the pore diameter is up to about 0.6 mu m.

16. The method according to claim 15, wherein the pore diameter is up to about 0.45 mu m.

17. The method according to claim 16, wherein the pore diameter is at the most about 0.2 mu m.

18. The method according to claim 6 wherein the aqueous medium in dialyzing step g) is water.

19. The method according to claim 10 wherein the aqueous medium in dialyzing step g) is water.

20. The method of claim 18 wherein the water is selected from the group consisting of tap water, demineralized water and distilled water.

21. The method of claim 19 wherein the water is selected from the group consisting of tap water, demineralized water and distilled water.

1/3,AB,CM/8 (Item 8 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02470422
Utility
NUCLEIC ACID PROBES FOR THE DETECTION OF LYME DISEASE SPIROCHETES

PATENT NO.: 5,466,577
ISSUED: November 14, 1995 (19951114)
INVENTOR(s): Weisburg, William G., Milford, MA (Massachusettes), US (United States of America)
ASSIGNEE(s): Amoco Corporation, (A U.S. Company or Corporation), US (United States of America)
[Assignee Code(s): 79752]
APPL. NO.: 8-144,212
FILED: October 27, 1993 (19931027)

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation of application Ser. No. 773,351, filed Oct. 8, 1991, now abandoned, which is a continuation of application Ser. No. 416,072, filed Oct. 2, 1989, now abandoned.

FULL TEXT: 982 lines

ABSTRACT

Nucleic acid probes are described for detecting bacteria capable of causing Lyme disease. These probes complement the ribosomal ribonucleic acid sequences unique to Borrelia spirochetes, and as such can detect the rRNA, rDNA, or polymerase chain reaction amplification products of these genes. These probes, plus the described amplification primers, can be used to detect the etiological agent of Lyme disease in human or veterinary samples, and for determining the infective potential of Ixodes ticks.

What is claimed is:

1. An isolated nucleic acid probe consisting of a nucleotide sequence identical or fully complementary to a region of the 16S rRNA of Borrelia burgdorferi bounded by nucleotide positions 63 to 106, 178 to 194, 416 to 450, 453 to 481, 829 to 866, 1122 to 1156, or 1414 to 1475, which nucleic acid probe preferentially hybridizes to the 16S rRNA or rDNA of Borrelia bacteria over the 16S rRNA or rDNA of non-Borrelia bacteria.

2. An isolated nucleic acid probe of claim 1, wherein said nucleotide sequence is identical or fully complementary to the nucleotide sequence of probe 1616 (SEQ ID NO: 1), 1617 (SEQ ID NO: 2), 1618 (SEQ ID NO: 3), 1619 (SEQ ID NO: 4), 1620 (SEQ ID NO: 5), 1621 (SEQ ID NO: 6), or 1622 (SEQ ID NO: 7).

3. An isolated nucleic acid probe consisting of a nucleotide sequence identical or fully complementary to the nucleotide sequence of probe 1643 (SEQ ID NO: 15) or 1637 (SEQ ID NO: 16).

4. A method for detecting Borrelia bacteria in a biological sample from a patient comprising the steps of:

a) processing said sample to yield nucleic acid;

b) contacting said nucleic acid with at least one nucleic acid probe of claim 1;

c) imposing hybridization conditions on the nucleic acid and said probe to allow said probe to hybridize to rRNA or rDNA of a Borrelia species, if present in said sample, to form nucleic acid complexes, and not to hybridize with rRNA or rDNA of a non-Borrelia organism; and

d) detecting said nucleic acid complexes as an indication of the presence of a Borrelia species in the sample.

5. The method of claim 4, wherein said nucleic acid probe of said contacting step consists of a nucleotide sequence identical or fully complementary to the nucleotide sequence of probe 1616 (SEQ ID NO: 1), 1617 (SEQ ID NO: 2), 1618 (SEQ ID NO: 3), 1619 (SEQ ID NO: 4), 1620 (SEQ ID NO: 5), 1621 (SEQ ID NO: 6), 1622 (SEQ ID NO: 7), 1643 (SEQ ID NO: 15), or 1637 (SEQ ID NO: 16).

6. The method of claim 4, wherein said contacting step comprises the use of probe 1643 (SEQ ID NO: 15) or probe 1637 (SEQ ID NO: 16) and said detecting step further comprises contacting said sample with a second nucleic acid probe consisting of a nucleotide sequence identical or fully complementary to the nucleotide sequence of probe 1616 (SEQ ID NO: 1), 1617 (SEQ ID NO: 2), 1618 (SEQ ID NO: 3), 1619 (SEQ ID NO: 4), 1620 (SEQ ID NO: 5), 1621 (SEQ ID NO: 6), or 1622 (SEQ ID NO: 7).

7. A new method of claim 4 further comprising the step of amplifying 16S rRNA or rDNA of said Borrelia species, if present, by polymerase chain reaction.

8. A set of nucleic acid probes comprising at least two probes, each probe consisting of a different nucleotide sequence identical or fully complementary to the nucleotide sequence of probe 1616 (SEQ ID NO: 1), 1617 (SEQ ID NO: 2), 1618 (SEQ ID NO: 3), 1619 (SEQ ID NO: 4), 1620 (SEQ ID NO: 5), 1621 (SEQ ID NO: 6), 1622 (SEQ ID NO: 7), 1643 (SEQ ID NO: 15), or 1637 (SEQ ID NO: 16).

9. A set of nucleic acid probes of claim 8, wherein said set consists of any one of probe sets 1616 (SEQ ID NO: 1) and 1617 (SEQ ID NO: 2), 1620 (SEQ ID NO: 5) and 1621 (SEQ ID NO: 6), or 1643 (SEQ ID NO: 15), 1620 (SEQ ID NO: 5), and any one of probes 1616 (SEQ ID NO: 1), 1617 (SEQ ID NO: 2), 1618 (SEQ ID NO: 3), and 1619 (SEQ ID NO: 4).

10. An isolated nucleic acid probe of claim 1, wherein said nucleotide sequence is identical or fully complementary to the nucleotide sequence of probe 1616 (SEQ ID NO: 1).

11. An isolated nucleic acid probe of claim 1, wherein said nucleotide sequence is identical or fully complementary to the nucleotide sequence of probe 1617 (SEQ ID NO: 2).

12. An isolated nucleic acid probe of claim 1, wherein said nucleotide sequence is identical or fully complementary to the nucleotide sequence of probe 1618 (SEQ ID NO: 3).

13. An isolated nucleic acid probe of claim 1, wherein said nucleotide sequence is identical or fully complementary to the nucleotide sequence of probe 1619 (SEQ ID NO: 4).

14. An isolated nucleic acid probe of claim 1, wherein said nucleotide sequence is identical or fully complementary to the nucleotide sequence of probe 1620 (SEQ ID NO: 5).

15. An isolated nucleic acid probe of claim 1, wherein said nucleotide sequence is identical or fully complementary to the nucleotide sequence of probe 1621 (SEQ ID NO: 6).

16. An isolated nucleic acid probe of claim 1, wherein said nucleotide sequence is identical or fully complementary to the nucleotide sequence of

probe 1622 (SEQ ID NO: 7).

1/3,AB,CM/9 (Item 9 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02436306
Utility
FLAGELLA-LESS BORRELIA

PATENT NO.: 5,436,000
ISSUED: July 25, 1995 (19950725)
INVENTOR(s): Barbour, Alan G., San Antonio, TX (Texas), US (United States of America)
Bundoc, Virgilio, San Antonio, TX (Texas), US (United States of America)
ASSIGNEE(s): University of Texas System, (A U.S. Company or Corporation), Austin, TX (Texas), US (United States of America)
[Assignee Code(s): 83960]
APPL. NO.: 7-641,143
FILED: January 11, 1991 (19910111)

FUNDING

Development of the present invention was aided in part by finding from The National Institute of Health, grant no. AI24424. Accordingly, the U.S. Government has a paid-up license and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided by the terms of Grant No. AI24424.

FULL TEXT: 1274 lines

ABSTRACT

This invention relates to flagella-less strains of Borrelia and to novel methods for use of the microorganisms as vaccines and in diagnostic assays. Although a preferred embodiment of the invention is directed to Borrelia burgdorferi, the present invention encompasses flagella-less strains of other microorganisms belonging to the genus Borrelia. Accordingly, with the aid of the disclosure, flagella-less mutants of other Borrelia species, e.g., B. coriacei, which causes epidemic bovine abortion, B. anserina, which causes avian spirochetosis, and B. recurrentis and other Borrelia species causative of relapsing fever, such as Borrelia hermsii, Borrelia turicatae, Borrelia duttoni, Borrelia persica, and Borrelia hispanica, can be prepared and used in accordance with the present invention and are within the scope of the invention. Therefore, a preferred embodiment comprises a composition of matter comprising a substantially pure preparation of a strain of a flagella-less microorganism belonging to the genus Borrelia.

We claim:

1. A substantially pure culture of flagella-less B. Burgdorferi as deposited with the American Type Culture Collection under Accession No. 55131.

1/3,AB,CM/10 (Item 10 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02400445
Utility
METHODS AND ANTIBODIES FOR THE IMMUNE CAPTURE AND DETECTION OF BORRELIA BURGDORFERI

[React with antigens from geographically diverse strains of borrelia burgdorferi, but do not react with antigens from related borrelia spirochetes; diagnosis of lyme disease]

PATENT NO.: 5,403,718
ISSUED: April 04, 1995 (19950404)
INVENTOR(s): Dorward, David W., 401 N. 7th St., Hamilton, MT (Montana), US (United States of America), 59840
Schwan, Tom G., 601 S. 5th St., Hamilton, MT (Montana), US (United States of America), 59840
Garon, Claude F., 904 Ponderosa Dr., Hamilton, MT (Montana), US (United States of America), 59840 68000]
EXTRA INFO: Assignment transaction [Reassigned], recorded August 11, 1992 (19920811)
APPL. NO.: 7-929,172
FILED: August 11, 1992 (19920811)
DISCLAIMER: June 08, 2010 (20100608)

This is a continuation-in-part of copending application U.S. Ser. No. 07-485,551, filed Feb. 27, 1990 now U.S. Pat. No. 5,217,872.

FULL TEXT: 1567 lines

ABSTRACT

The invention relates to novel antigens associated with Borrelia burgdorferi which are exported (or shed) in vivo and whose detection is a means of diagnosing Lyme disease. The antigens are extracellular membrane vesicles and other bioproducts including the major extracellular protein. The invention further provides antibodies, monoclonal and/or polyclonal, labeled and/or unlabeled, that react with the antigens. The invention relates to a method for immune capture of specific microorganisms for their subsequent cultivation. The invention is also directed to a method of diagnosing Lyme disease by detecting the antigens in a biological sample taken from a host using the antibodies in conventional immunoassay formats. The invention further relates to kits, for the diagnosis of Lyme disease, comprising the antibodies and ancillary reagents. The advantage of the antibodies used in the invention is that they react with the antigens from geographically diverse strains of Borrelia burgdorferi, but do not react with antigens from related Borrelia spirochetes.

What is claimed is:

1. A method of detecting Borrelia burgdorferi or Borrelia burgdorferi antigens in a sample, said method comprising:
a. providing:
i) a fluid or tissue sample;
ii) purified polyclonal antibodies or mixtures of monoclonal antibodies, said antibodies raised against the extracellular membrane vesicles exported from Borrelia burgdorferi; and,
iii) purified antibodies or antigen-binding fragments of said antibodies raised against the major extracellular protein exported from Borrelia burdorferi having a molecular weight of approximately 83 kDa;
b. contacting said sample with said antibodies of step a(ii) and said antibodies and step a(iii), under conditions in which ternary immune complexes will form among said antibodies of steps a(ii) and a(iii) and any antigens associated with Borrelia burdorferi that may be present in said sample; and
c. detecting the presence of said ternary immune complexes as a means of detecting Borrelia brugdorferi or Borrelia burgdorferi antigens.

2. Purified antibodies or antigen-binding fragments of said antibodies raised against purified extracellular membrane vesicles exported from Borrelia burgdorferi.

02380543

Utility

DIAGNOSTIC **ASSAY** FOR **LYME** DISEASE

[Preparing mixture of patient's serum and an inoculum of Borrelia organisms; incubation; determining number of viable organisms]

PATENT NO.: 5,385,826
ISSUED: January 31, 1995 (19950131)
INVENTOR(s): Schell, Ronald F., Madison, WI (Wisconsin), US (United States of America)
Callister, Steven M., Onalaska, WI (Wisconsin), US (United States of America)
ASSIGNEE(s): Gundersen Medical Foundation, Ltd, (A U.S. Company or Corporation), Lacrosse, WI (Wisconsin), US (United States of America)
[Assignee Code(s): 35338]
APPL. NO.: 8-88,590
FILED: August 09, 1993 (19930809)

This application is a continuation of application Ser. No. 07-605,798, filed Oct. 31, 1990, now abandoned, which was a continuation-in-part of application Ser. No. 07-341,459, filed Apr. 21, 1989, now abandoned.

FULL TEXT: 570 lines

ABSTRACT

A method of performing an assay to determine whether a patient has been exposed to or infected by Borrelia burgdorferi is disclosed which comprises collecting serum from the patient; preparing a sample mixture comprising a portion of the patient's serum and an inoculum of viable Borrelia burgdorferi organisms; incubating the sample mixture; determining the number of viable organisms remaining in the sample mixture after incubation; and comparing the number with the quantity of viable organisms remaining in a control. An assay kit is also disclosed which is useful for determining whether a patient has been exposed to or infected by Borrelia burgdorferi. The kit contains reagents necessary to practice the assay method disclosed herein. In its broadest form, the kit comprises an inoculum of viable Borrelia burgdorferi organisms. The kit can also contain an aliquot of normal serum, an aliquot of BSK medium and/or an aliquot of complement. Other reagents, tubes and other materials can also be included in the kits.

We claim:

1. A method of performing an assay to determine whether a patient has been exposed to Borrelia burgdorferi, said method comprising:
collecting serum from a patient which may have been exposed to Borrelia burgdorferi;
preparing a sample mixture, said sample mixture comprising a portion of said patient's serum and an inoculum of viable Borrelia burgdorferi organisms;
incubating said sample mixture;
determining the number of viable organisms remaining in said sample mixture after incubation; and
comparing said number with the quantity of viable organisms remaining in a control mixture.

2. The method of claim 1 wherein said serum is heat inactivated before preparation of said sample mixture.

3. The method of claim 1 wherein said Borrelia burgdorferi organisms are

of a strain selected from the group consisting of strain 297, strain B31 and a strain indigenous to the area in which said patient is suspected to have been exposed to Borrelia burgdorferi.

4. The method of claim 3 wherein said Borrelia burgdorferi organisms are of strain 297.

5. The method of claim 3 wherein said Borrelia burgdorferi organisms are of a strain indigenous to the area in which said patient is suspected to have been exposed to Borrelia burgdorferi.

6. The method of claim 1 wherein said Borrelia burgdorferi organisms are properly aged organisms.

7. The method of claim 1 wherein said sample mixture and said control mixture are incubated at 32 degree(s) C.

8. The method of claim 7 wherein said sample mixture is incubated for at least about 30 minutes,

9. The method of claim 8 wherein said sample mixture is incubated for about 6 hours,

10. The method of claim 8 wherein said sample mixture is incubated for about.18 hours,

11. The method of claim 1 wherein said number of viable organisms remaining in said sample mixture and said quantity of viable organisms remaining in said control mixture are determined by counting viable Borrelia burgdorferi organisms under a microscope.

12. The method of claim 1 wherein said patient is human.

13. The method of claim 1 wherein said control mixture is prepared by mixing normal serum and an inoculum of viable Borrelia burgdorferi organisms, and wherein said control mixture is incubated with said sample mixture.

14. The method of claim 1 wherein said number of viable organisms remaining in said sample mixture and said quantity of viable organisms remaining in said control mixture are determined by measurement of uptake of sup 3 H-adenine.

15. The method of claim 1 wherein said number of viable organisms remaining in said sample mixture and said quantity of viable organisms remaining in said control mixture are determined by flow cytometry or Coulter counter.

16. A method of performing an assay to determine whether a patient has been exposed to Borrelia burgdorferi, said method comprising:

collecting serum from a patient which may have been exposed to Borrelia burgdorferi;

heat inactivating said serum;

preparing a sample mixture, said sample mixture comprising a portion of said patient's heat-inactivated serum and an inoculum of viable Borrelia burgdorferi organisms;

incubating said sample mixture;

determining the number of viable organisms remaining in said sample mixture after incubation; and

comparing said number with the quantity of viable organisms remaining in a control mixture.

17. The method of claim 16 wherein said Borrelia burgdorferi organisms are of a strain selected from the group consisting of strain 297. strain B31 and a strain indigenous to the area in which said patient is suspected to have been exposed to Borrelia burgdorferi.

18. The method of claim 17 wherein said Borrelia burgdorferi organisms are of strain 297.

19. The method of claim 17 wherein said Borrelia burgdorferi organisms are of a strain indigenous to the area in which said patient is suspected to have been exposed to Borrelia burgdorferi.

20. The method of claim 16 wherein said Borrelia burgdorferi organisms are properly aged organisms.

21. The method of claim 16 wherein said sample mixture and said control mixture are incubated at 32 degree(s) C.

22. The method of claim 21 wherein said sample mixture is incubated for at least about 30 minutes.

23. The method of claim 22 wherein said sample mixture is incubated for about 6 hours.

24. The method of claim 22 wherein said sample mixture is incubated for about 18 hours.

25. The method of claim 16 wherein said number of viable organisms remaining in said sample mixture and said quantity of viable organisms remaining in said control mixture are determined by counting viable Borrelia burgdorferi organisms in said sample mixture and in said control mixture using a microscope.

26. The method of claim 16 wherein said patient is human.

27. The method of claim 16 wherein said control mixture is prepared by mixing normal serum and an inoculum of viable Borrelia burgdorferi organisms, and wherein said control mixture is incubated with said sample mixture.

28. The method of claim 16 wherein said number of viable organisms remaining in said sample mixture and said quantity of viable organisms remaining in said control mixture are determined by measurement of uptake of sup 3 H-adenine.

29. The method of claim 16 wherein said number of viable organisms remaining in said sample mixture and said quantity of viable organisms remaining in said control mixture are determined by flow cytometry or Coulter counter.

30. A method of performing an assay to determine whether a patient has been exposed to Borrelia burgdorferi, said method comprising:
collecting serum from a patient which may have been exposed to Borrelia burgdorferi;
preparing a sample mixture, said sample mixture comprising a portion of said patient's serum, an inoculum of viable Borrelia burgdorferi organisms, and added complement;
incubating said sample mixture;
determining the number of viable organisms remaining in said sample mixture after incubation; and
comparing said number with the quantity of viable organisms remaining in a control mixture.

31. A method of performing an assay to determine whether a patient has been exposed to Borrelia burgdorferi, said method comprising:
collecting serum from a patient which may have been exposed to Borrelia burgdorferi;
heat inactivating said serum;
preparing a sample mixture, said sample mixture comprising a portion of said patient's heat-inactivated serum, an inoculum of viable Borrelia burgdorferi organisms, and added complement;
incubating said sample mixture;
determining the number of viable organisms remaining in said sample mixture after incubation; and
comparing said number with the quantity of viable organisms remaining in a control mixture.

32. The method of claim 1 wherein said sample mixture further comprises added complement.

33. The method of claim 16 wherein said sample mixture further comprises added complement.

34. The method of claim 1 wherein said sample mixture further comprises BSK medium.

35. The method of claim 16 wherein said sample mixture further comprises BSK medium.

1/3,AB,CM/12 (Item 12 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02363902

Utility
SOLID PHASE ASSAY FOR UREA
[Applying solution containing urea, pH-dependent reducing agent and tetrazolium salt which will be reduced to colored formazan dye(if urea is present) to support containing adsorbed urease]

PATENT NO.: 5,370,994
ISSUED: December 06, 1994 (19941206)
INVENTOR(s): Stewart, Thomas N., Durham, NC (North Carolina), US (United States of America)
Vonk, Glenn P., Fuquay-Varina, NC (North Carolina), US (United States of America)
Mapes, James P., Raleigh, NC (North Carolina), US (United States of America)
ASSIGNEE(s): Becton, Dickinson and Company, (A U.S. Company or Corporation), Franklin Lakes, NJ (New Jersey), US (United States of America)
[Assignee Code(s): 8488]
APPL. NO.: 8-165,220
FILED: December 10, 1993 (19931210)

This is a division of application Ser. No. 07-851,602, filed Mar. 16, 1992 now U.S. Pat. No. 5,328,831.

FULL TEXT: 354 lines

ABSTRACT

A method for detecting urea in a liquid sample in which urease is adsorbed on a solid support such as a membrane and contacted with a solution suspected of containing urea, a pH-dependent reducing agent and a tetrazolium salt. When urea is present in the solution, the adsorbed urease converts it to ammonia, thus raising the pH and causing the pH-dependent reducing agent to reduce the tetrazolium salt to an insoluble colored formazan. The formazan precipitates as a detectable spot on the solid support, indicating the presence of urea in the liquid sample.

What is claimed is:

1. A method for detecting urea comprising:
a) adsorbing urease onto a solid support;
b) contacting the support with
i) a solution suspected of containing urea;
ii) a pH-dependent reducing agent selected from the group consisting of indoxyl, indoxyl butyrate, sodium ascorbate and ascorbic acid, and
iii) a tetrazolium salt such that the tetrazolium salt is reduced to a colored formazan which forms a colored spot on the support if urea is present in the solution.

2. The method of claim 1 wherein the tetrazolium salt is selected from the group consisting of iodonitrotetrazolium violet and nitrobluetetrazolium chloride.

1/3,AB,CM/13 (Item 13 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02345809

Utility
NON-RADIOACTIVE METHOD FOR DETECTING A LABELLED SEGMENT AND A SOLUTION OR

COMPOSITION THEREFOR
[Reacting complex with bromo-chloro-indolyl phosphate, phenazine methosulfate, and dimethylthiazole diphenyl tetrazolium to produce colored formazan]

PATENT NO.: 5,354,658
ISSUED: October 11, 1994 (19941011)
INVENTOR(s): Wright, Dennis, 2925 E. Wisconsin Apt. D., Great Lakes, IL (Illinois), US (United States of America), 60088 68000]
APPL. NO.: 8-10,344
FILED: January 28, 1993 (19930128)

FULL TEXT: 762 lines

ABSTRACT

A non-radioactive method of detecting a ligand and antiligand complex labelled with alkaline phosphatase or a tracer having alkaline phosphatase conjugated thereto comprises reacting the complex with bromo-chloro-indolyl phosphate (BCIP), phenazine methosulfate (PMS) and dimethylthiazol diphenyl tetrazolium (MTT) and allowing the reaction to proceed to produce a colored formazan or a color change indicative of the presence of the labelled complex. A solution or composition of bromo-chloro-indolyl phosphate (BCIP), phenazine methosulfate (PMS) and dimethylthiazol diphenyl tetrazolium (MTT), as well as a test kit including the same, is also provided for carrying out the chromogenic method of detection.

I claim:

1. A non-radioactive method of detecting a ligand and antiligand complex labelled with alkaline phosphatase or a tracer having alkaline phosphatase conjugated thereto comprising reacting said complex with bromo-chloro-indolyl phosphate, phenazine methosulfate and dimethylthiazol diphenyl tetrazolium and allowing the reaction to proceed to produce a colored formazan or a color change indicative of the presence of said labelled complex.

2. The method of claim 1 wherein said ligand is selected from the group consisting of an antigen, an analyte, a protein, an antibody, an antibody complex, and a hapten.

3. The method of claim 1 wherein said antiligand is selected from the group consisting of an antigen, an analyte, a protein, an antibody, an antibody complex, and a hapten.

4. The method of claim 2 wherein said ligand is an antigen and said antiligand is an antibody specific for said antigen.

5. The method of claim 2 wherein said ligand is a hapten and said antiligand is an antibody specific for said hapten.

6. The method of claim 2 wherein said ligand is an antibody and said antiligand is an antigen specific for said antibody.

7. The method of claim 2 wherein said ligand is a protein and said antiligand is an antibody specific for said protein.

8. The method of claim 2 wherein said ligand is a nucleic acid and said antiligand is a complimentary nucleic acid specific for said nucleic acid.

9. The method of claim 2 wherein said ligand is an antibody complex and said antiligand is an antigen specific for said antibody complex.

10. The method of claim 1 wherein a ligand directly labelled with alkaline phosphatase and a tracer selected from the group consisting of an antigen, an analyte, a protein, an antibody, an antibody complex, and a hapten compete for binding sites on said antiligand.

11. The method of claim 1 wherein a ligand selected from the group consisting of an antigen, an analyte, a protein, an antibody, an antibody complex, and a hapten and a tracer having alkaline phosphatase conjugated thereto compete for binding sites on said antiligand.

12. The method of claim 1 wherein the amount, intensity, or degree of produced colored formazan or color change is determined visually or instrumentally.

13. The method of claim 1 wherein the complex is reacted with a mixture containing in combination bromo-chloro-indolyl phosphate, phenazine methosulfate and dimethylthiazol diphenyl tetrazolium.

14. The method of claim 13 wherein said mixture further includes a buffer.

15. The method of claim 14 wherein said buffer is distilled water.

16. The method of claim 14 wherein said buffer is a mixture in solution of Tris-HCl or Tris-base, sodium chloride, and magnesium chloride.

17. The method of claim 14 wherein the buffer has a pH of 7 to 11.

18. The method of claim 14 wherein the buffer has a pH of 9.5.

19. A solution for the detection of a ligand and antiligand complex labelled with alkaline phosphatase or a tracer having alkaline phosphatase conjugated thereto in a sample to be tested comprising a mixture of bromo-chloro-indolyl phosphate, phenazine methosulfate and dimethylthiazol diphenyl tetrazolium which when added to said test sample is capable of producing a colored formazan or a color change indicative of the presence of said labelled complex.

20. The solution of claim 19 containing approximately equal amounts of phenazine methosulfate and dimethylthiazol diphenyl tetrazolium in combination with an excess of bromo-chloro-indolyl phosphate.

21. The solution of claim 19 wherein the ratio of bromo-chloro-indolyl phosphate, phenazine methosulfate and dimethylthiazol diphenyl tetrazolium respectively in said solution is about 6:1:1 by weight.

22. The solution of claim 19 including from 35 to 50 microliters of bromo-chloro-indolyl phosphate from a 50 mg/ml aqueous solution, from 70 to 100 microliters of phenazine methosulfate from a 10 mM aqueous solution and from 70 to 100 microliters of dimethylthiazol diphenyl tetrazolium from a 10 mM aqueous solution.

23. The solution of claim 19 capable of producing a sufficient colored formazan or a color change indicative of the presence or concentration of said complex within fifteen minutes of contacting at ambient temperature said test sample.

24. The solution of claim 19 wherein the amount, intensity, or degree of the produced colored formazan or color change is sufficient to accurately determine visually or instrumentally the presence or concentration of said complex in said test sample.

25. The solution of claim 19 further including a buffer.

26. The solution of claim 25 wherein the buffer is distilled water.

27. The solution of claim 25 wherein the buffer is a mixture in solution of Tris-HCl or Tris-base, sodium chloride, and magnesium chloride.

28. The solution of claim 25 wherein the buffer has a pH of 7 to 11.

29. The solution of claim 25 wherein the buffer has a pH of 9.5.

30. A composition for the detection of a ligand and antiligand complex

labelled with alkaline phosphatase or a tracer having alkaline phosphatase conjugated thereto in a sample to be tested comprising a mixture of bromo-chloro-indolyl phosphate, phenazine methosulfate and dimethylthiazol diphenyl tetrazolium which when dissolved in solution and added to said test sample is capable of producing a colored formazan or a color change indicative of the presence of said labelled complex.

31. The composition of claim 30 in powder form.

32. The composition of claim 30 in solid form.

33. The composition of claim 30 further including an inert carrier.

34. The composition of claim 30 wherein the inert carrier is soluble in water.

35. The composition of claim 30 wherein the inert carrier is mannitol.

36. The composition of claim 30 containing approximately equal amounts of phenazine methosulfate and dimethylthiazol diphenyl tetrazolium in combination with an excess of bromo-chloro-indolyl phosphate.

37. The composition of claim 30 wherein the ratio of bromo-chloro-indolyl phosphate, phenazine methosulfate and dimethylthiazol diphenyl tetrazolium respectively in said composition is about 6:1:1 by weight.

38. A kit of materials for performing the method of detection of a ligand and antiligand complex labelled with alkaline phosphatase or a tracer having alkaline phosphatase conjugated thereto according to claim 1, comprising a vial or packet of bromo-chloro-indolyl phosphate, phenazine methosulfate and dimethylthiazol diphenyl tetrazolium in an amount sufficient, when reacted with said labelled complex, to produce a colored formazan or a color change indicative of the presence of said labelled complex.

1/3,AB,CM/14 (Item 14 from file: 654)

02317290

Utility
SUBSTRATE COMPOSITION FOR SOLID PHASE UREASE IMMUNOASSAY
[Solid phase enzyme analysis]

PATENT NO.: 5,328,831
ISSUED: July 12, 1994 (19940712)
INVENTOR(s): Stewart, Thomas N., Durham, NC (North Carolina), US (United States of America)
Vonk, Glenn P., Fuquay-Varina, NC (North Carolina), US (United States of America)
Mapes, James P., Raleigh, NC (North Carolina), US (United States of America)
ASSIGNEE(s): Becton, Dickinson & Company, (A U.S. Company or Corporation), Franklin Lakes, NJ (New Jersey), US (United States of America)
[Assignee Code(s): 8488]
APPL. NO.: 7-851,602
FILED: March 16, 1992 (19920316)

This is a division of application Ser. No. 07-528,526, filed May 25, 1990, now U.S. Pat. No. 5,139,934.

FULL TEXT: 360 lines

ABSTRACT

APPL. NO.: 7-943,515
FILED: September 11, 1992 (19920911)

FULL TEXT: 309 lines

ABSTRACT

Disclosed is a method for preventing the transmission of an ectoparasite-borne pathogen to a mammal exposed to predation by the ectoparasite, wherein a time delay exists between attachment of the ectoparasite to the mammal and transmission of the pathogen from the ectoparasite to the mammal. The method comprises applying to the mammal an ectoparacide to kill the ectoparasite in place on the mammal, without necessarily finding or mechanically detaching the ectoparasite, at a time after the exposure of the mammal to the ectoparasite, and within the time delay, thereby preventing transmission of the pathogen from the ectoparasite to the mammal.

What is claimed is:

1. A method for preventing the transmission of a tick-borne pathogen to a mammal exposed to predation by a tick infected with said pathogen, wherein a time delay exists between attachment of said infected tick to said mammal and transmission of said pathogen from said infected tick to said mammal, the method comprising:
applying to said mammal an ectoparacide to kill said infected tick, attached to said mammal, without necessarily finding or mechanically detaching said infected tick, at a time after the exposure of said mammal to said infected tick, and within said time delay, thereby to prevent transmission of said pathogen from said infected tick to said mammal, and
repeating said applying step within said time delay after each said exposure.

2. The method of claim 1 wherein said ectoparacide comprises a soap, and wherein said applying step comprises washing said mammal with said soap.

3. The method of claim 1 wherein said pathogen is an organism selected from the group consisting of Borellia burgdorferi, Babesia microti, Rickettsia rickettsi, R. australis and R. tsutsugamushi.

4. The method of claim 1 wherein said tick is Ixodes dammini;
wherein said pathogen is Borrelia burgdorferi; and
wherein said ectoparacide is applied within approximately 24 hours following exposure of said mammal to said tick, Ixodes dammini.

5. The method of claim 4, wherein said applying step is repeated periodically approximately every 24 hours throughout the duration of exposure of said mammal to said tick, Ixodes dammini.

6. The method of claims 4 or 5 wherein said ectoparacide comprises a soap containing a pyrethroid; and
wherein said applying step comprises washing said mammal with said soap.

7. The method of claim 6 wherein said pyrethroid is permethrin.

8. The method of claims 4 or 5 wherein said ectoparacide comprises a composition selected from the group consisting of a pyrethrum, an ectoparacidal fatty acid, an ectroparacidal organophosphate insecticide and combinations thereof.

9. The method of claim 1 wherein said ectoparacide comprises a vehicle selected from the group consisting of a cream and a soap.

10. The method of claim 1 wherein said applying step comprises spraying said mammal with said ectoparacide.

A substrate composition for use in a solid phase enzyme assay for urease or in a solid phase enzyme immunoassay which includes urease as a label. The substrate composition includes a compound converted by urease to ammonia and a pH dependent reducing agent which reduces a tetrazolium salt when the pH of the medium has been raised by the ammonia produced. The tetrazolium salt may optionally be included in the substrate composition. Reduction of the tetrazolium salt produces a colored insoluble formazan which precipitates on the solid phase as an indication of the presence of urease.

What is claimed is:

1. A urease substrate composition comprising;
a) a first component selected from the group consisting of urea, substituted ureas and simple amides;
b) a second component selected from the group consisting of indoxyl, indoxyl butyrate, sodium ascorbate and ascorbic acid, and;
c) optionally a tetrazolium salt;

wherein the composition has an acidic pH which prevents reduction of the tetrazolium salt by the second component, if the tetrazolium salt is present, and wherein the first and second components are present in amounts such that cleavage of the first component by urease increases the pH and allows the second component to reduce the tetrazolium salt if present to an insoluble formazan.

2. The composition of claim 1 wherein the first component is selected from the group consisting of urea, N-methylurea, semicarbazide, acetamide and formamide.

3. The composition of claim 2 wherein the first component is about 25 mM urea and the second component is about 20 mM sodium ascorbate.

4. The composition of claim 3 wherein the tetrazolium salt is selected from the group consisting of iodonitrotetrazolium violet and nitrobluetetrazolium chloride.
5. The composition of claim 1 wherein the first component is about 25 mM urea and the second component is about 1 mM indoxyl butyrate.

6. The composition of claim 5 which comprises a tetrazolium salt selected from the group consisting of iodonitrotetrazolium violet and nitrobluetetrazolium chloride.

7. The composition of claim 1 which comprises a tetrazolium salt.

8. The composition of claim 7 wherein the tetrazolium salt is selected from the group consisting of iodonitrotetrazolium violet and nitrobluetetrazolium chloride.

1/3,AB,CM/15 (Item 15 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02266802
Utility
METHOD FOR THE PREVENTION OF ECTOPARASITE-MEDIATED PATHOGEN TRANSMISSION
[Applying ectoparasiticide after exposure, within time delay from attachment to transmission, without detaching tick]

PATENT NO.: 5,283,259
ISSUED: February 01, 1994 (19940201)
INVENTOR(s): Mather, Thomas N., Wakefield, RI (Rhode Island), US (United States of America)
ASSIGNEE(s): The President & Fellows of Harvard College, (A U.S. Company or Corporation), Cambridge, MA (Massachusettes), US (United States of America)
[Assignee Code(s): 542]

02262999
Utility

SENSITIVE DIAGNOSTIC TEST FOR LYME DISEASE
[Using polypeptide]

PATENT NO.: 5,279,938
ISSUED: January 18, 1994 (19940118)
INVENTOR(s): Rosa, Patricia A., Hamilton, MT (Montana), US (United States of America)
ASSIGNEE(s): The United States of America as represented by the Department of Health & Human Services, (A U.S. Government Agency), Washington, DC (District of Columbia), US (United States of America)
[Assignee Code(s): 6814]
APPL. NO.: 7-885,077
FILED: May 18, 1992 (19920518)

This is a continuation of co-pending application Ser. No. 07-361,850, filed on Jun. 5, 1989, now abandoned.

FULL TEXT: 550 lines

ABSTRACT

The nucleotide sequence of a recombinant clone containing a specific segment of Borrelia burgdorferi DNA which enables the identification of the spirochetes causing Lyme disease has been provided. A diagnostic kit containing oligonucleotide primers derived from this sequence, suitable for the detection of Borrelia burgdoferi in a PCR assay, as well as the cloned DNA of the present invention, allows the detection of Lyme disease with sensitivity and specificity not heretofore attained by any other test.

What is claimed is:

1. An isolated DNA segment consisting of the DNA sequence [See structure in original document] which hybridizes with DNA of Borrelia burgdorferi origin, and which does not cross hybridize with other Borrelia species.

2. A cloned DNA segment of the sequence of claim 1 and fragments derived from said sequence and specifically hybridizable therewith.

3. The fragments of claim 2 consisting of the sequences [See structure in original document]

4. The cloned DNA of claim 2 having ATCC deposit no. 67984.

5. A diagnostic kit for the detection of Borrelia burgdorferi DNA, comprising a first container containing the nucleotide sequence, having the sequence of claim 1, and a second container containing oligonucleotide primers derived from said sequence for employing in the polymerase chain reaction assay.

6. A method for detecting presence or absence of Borrelia burgdorferi in a specimen comprising:

a) purifying the DNA from the specimen suspected of Borrelia burgdorferi infection;

b) adding the DNA obtained from step (a) to a polymerase chain reaction reaction mixture containing primers for amplifying Borrelia burgdorferi-specific DNA of the sequence of claim 1;

c) amplifying by polymerase chain reaction;

d) testing aliquots of the polymerase chain reaction reaction reaction product from step (c) for the presence or absence of Borrelia burgdorferi-specific amplified DNA by conventional means including gel electrophoresis and hybridization with a labeled probe internal to the amplified fragment, wherein a positive detection of said Borrelia

burgdorferi-specific DNA being indicative of the presence of Borrelia burgdorferi in said specimen.

1/3,AB,CM/17 (Item 17 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02225966
Utility
VIRULENCE ASSOCIATED PROTEINS IN BORRELIA BURGDORFERI (BB)
[Detection of Lyme disease]

PATENT NO.: 5,246,844
ISSUED: September 21, 1993 (19930921)
INVENTOR(s): Norris, Steven J., Houston, TX (Texas), US (United States of America)
Barbour, Alan G., San Antonio, TX (Texas), US (United States of America)
ASSIGNEE(s): Board of Regents, The University of Texas System, (A U.S. Company or Corporation), Austin, TX (Texas), US (United States of America)
[Assignee Code(s): 83960]
APPL. NO.: 7-781,355
FILED: October 22, 1991 (19911022)

The United States Government may have certain rights in the present invention pursuant to Grant No. AI 24424 and Grant No. AI 29731 awarded by the National Institutes of Health.

FULL TEXT: 1588 lines

ABSTRACT

The invention relates to a DNA segment encoding a Borrelia burgdorferi antigenic polypeptide. The invention also relates to a purified 30 kDa polypeptide isolated from a virulent strain of B. burgdorferi and to epitopic segments of the polypeptide with immunogenic potential. The 30 kDa protein provides a route for the development of immunodiagnostics for Lyme disease and related disorders. The 30 kDa protein and related amino acid and DNA sequences may also be used for the immunization, for the detection of B. burgdorferi in human or animal tissues or body fluids, and also for the generation of specific antibodies for use in diagnosis, epidemiology, and prevention of Lyme disease.

What is claimed is:

1. A DNA segment which comprises at least a 20 base pair segment according to SEQ ID NO:1 and which will bind to the complement of said sequence under high stringency conditions.

2. The DNA segment of claim 1 which comprises at least a 30 base pair segment corresponding to the DNA segment defined by SEQ ID NO:1.

3. The DNA segment of claim 1 which comprises at least a 40 base pair segment corresponding to the DNA segment defined by SEQ ID NO: 1.

4. The DNA segment of claim 1 which corresponds to the DNA sequence of SEQ ID NO:1.

5. The DNA segment of claim 1 wherein the DNA segment encodes an amino acid sequence comprising the sequencing from the amino acid Asp at position 23 through the amino acid Gln at position 54 according to SEQ ID NO:2.

6. The DNA segment of claim 1 wherein the DNA segment encodes an amino acid sequence comprising the sequence from the amino acid Leu at position

64 through the amino acid Ala at position 87 according to SEQ ID NO:2.

7. The DNA segment of claim 1 wherein the DNA segment encodes an amino acid sequence comprising the sequence from the amino acid Asp at position 106 through the amino acid Asn at position 114 according to SEQ ID NO:2.

8. The DNA segment of claim 1 wherein the DNA segment encodes an amino acid sequence comprising the sequence from the amino acid Lys at position 128 through the amino acid Ser at position 133 according to SEQ ID NO:2.

9. The DNA segment of claim 1 wherein the DNA segment encodes an amino acid sequence comprising the sequence from the amino acid Ala at position 152 through the amino acid Ala at position 188 according to SEQ ID NO:2.

10. The DNA segment of claim 1 wherein the DNA segment encodes an amino acid sequence comprising the sequence from the amino acid Asn at position 208 through the amino acid Lys at position 226 according to SEQ ID NO: 2.

11. A recombinant vector comprising the DNA segment of any one of claims 1, 3-4 or 6.

12. The recombinant vector of claim 11 wherein the DNA segment encodes an antigenic protein expressed in a low-passage, virulent strain of B. burgdorferi.

13. A recombinant cell comprising an extrachromosomal DNA segment in accordance with ATCC deposited plasmid accession number 75304 or ATCC accession number 75305.

14. The recombinant cell of claim 13 wherein the extrachromosomal DNA expresses a polypeptide encoded by the DNA of any of claims 1, 3 or 4.

15. A recombinant cell which is transformed with plasmic ATCC Accession No. 75304 and plasmid ATCC Accession No. 75305.

16. The recombinant cell of claim 15 wherein the cell is B. burgdorferi or E. coli.

17. A method of preparing transformed bacterial host cells, comprising the steps:
selecting a suitable bacterial host cells;
preparing a vector or plasmid containing the DNA segment of any of claims 1, 3-4 or 6; and
transforming the selected bacterial host cell.

18. The method of claim 17 wherein the plasmid or vector is capable of transforming the selected bacterial host cell to express a B. burgdorferi polypeptide encoded by the DNA of any of claims 1, 3-4 or 6.

19. The method of claim 18 wherein the expressed polypeptide is encoded by the DNA in accordance with any of claims 1, 3-4 or 6.

20. A B. burgdorferi transformant prepared by the method of claim 17.

21. A set of primers capable of priming amplification of the DNA according to SEQ ID NO:1.

22. A kit for the detection of Borrelia burgdorferi nucleic acids in a sample, the kit comprising a nucleic acid probe specific for the 30 kDa gene, together with means for detecting a specific hydridization between said probe and Bb nucleic acid.

1/3,AB,CM/18 (Item 18 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02191473
Utility

METHOD FOR DETECTION OF BORRELIA BURGDORFERI ANTIGENS
[Diagnosing Lymes disease]

PATENT NO.: 5,217,872
ISSUED: June 08, 1993 (19930608)
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ASSIGNEE(s): The United States of America as represented by the Department of Health and Human Services, (A U.S. Government Agency), Washington, DC (District of Columbia), US (United States of America)
[Assignee Code(s): 6814]
APPL. NO.: 7-485,551
FILED: February 27, 1990 (19900227)
FULL TEXT: 1102 lines

ABSTRACT

The invention relates to novel antigens associated with Borrelia burgdorferi which are exported (or shed) in vivo and whose detection is a means of diagnosing Lyme disease. The antigens are extracellular membrane vesicles and other bioproducts including the major extracellular protein. The invention further provides antibodies, monoclonal and/or polyclonal, labeled and/or unlabeled, that react with the antigens. The invention is also directed to a method of diagnosing Lyme disease by detecting the antigens in a biological sample taken from a host using the antibodies in conventional immunoassay formats. The invention further relates to kits, for the diagnosis of Lyme disease, comprising the antibodies and ancillary reagents. The advantage of the antibodies used in the invention is that they react with the antigens from geographically diverse strains of Borrelia burgdorferi, but do not react with antigens from related Borrelia spirochetes.

What is claimed is:

1. Purified antibodies or antigen-binding fragments of said antibodies raised against a purified major extracellular protein antigen which is exported from Borrelia burgdorferi, said purified major extracellular protein antigen having a molecular weight of 83 kilodaltons (kDa).

2. The isolated antibodies or antibody fragments of claim 1, wherein said antibodies are polyclonal.

3. The antibodies or antibody fragments of claim 1, wherein said antibodies are monoclonal.

4. The antibodies or antibody fragments of claim 1, wherein said antibodies are labeled with a detectable label.

5. The antibodies or antibody fragments of claim 4, wherein said detectable label is chosen from the group consisting of radioisotopes, enzymes, fluorophores and colloidal metals.

6. A method of diagnosing Lyme disease in a mammal, which method comprises:

i) obtaining a fluid or tissue sample from said mammal,

ii) contacting said sample with the antibodies or antigen binding fragments of said antibodies of claim 1, under conditions in which immune complexes will form between said antibodies or fragments and any antigens associated with Borrelia burgdorferi that may be present in said sample, and

iii) detecting the presence of said immune complexes as a means of diagnosing Lyme disease in said mammal.

7. The method of claim 6, wherein said antibodies are labeled with a detectable label.

8. The method of claim 7, wherein said detectable label is chosen from the group consisting of radioisotopes, enzymes, fluorophores and colloidal metals.

9. Purified antibodies or antigen-binding fragments of said antibodies raised against purified extracellular membrane vesicles exported from Borrelia burgdorferi, said antibodies being polyclonal.

10. A method of detecting Borrelia burgdorfer, or Borrelia burdorferi exported antigens in a sample, said method comprising:
A) providing:
i) a fluid or tissue sample,
ii) purified polyclonal antibodies or antigen-binding fragments of said polyclonal antibodies raised against the extracellular membrane vesicles exported from Borrelia burgdorferi, and
iii) purified antibodies or antigen-binding fragments of said antibodies raised against the major extracellular protein antigen exported from Borrelia burgdorferi, said protein antigen having a molecular weight of approximately 83 kDa;
B) contacting said sample with said polyclonal antibodies of step A (ii) and said antibodies of step A (iii), under conditions in which ternary immune complexes will form among said antibodies of steps A (ii) and A (iii) and any antigens associated with Borrelia burgdorferi that may be present in same sample; and
C) detecting the presence of said ternary immune complexes as a means of detecting Borrelia burgdorferi or Borrelia burgdorferi exported antigens.

11. The method of claim 10, wherein said polyclonal antibodies or antibody fragments of step A(ii) are bound to a solid substrate.

12. The method of claim 11, wherein said antibodies or antibody fragments of step A(iii) are labeled with a detectable label.

13. The method of claim 12, wherein said detectable label is chosen from the group consisting of radioisotopes, enzymes, fluorophores and colloidal metals.

14. The method of claim 11, wherein said antibodies of step A(iii) are not labeled with a detectable label and step C comprises contacting said ternary immune complexes with a specific binding protein which specifically binds to said antibodies of step A(iii), said specific binding protein being labeled with a detectable label.

15. The method of claim 14, wherein said specific binding protein is an antibody or protein A.

16. The method of claim 15, wherein said detectable label is chosen from the group consisting of radioisotopes, enzymes, fluorophores and colloidal metals.

17. The method of claim 16, wherein said specific binding protein is protein A and said polyclonal antibodies or antibody fragments of step A(iii) are F(ab') sub 2 antibody fragments.

18. The method of claim 10, wherein said sample is a fluid or tissue sample taken from a mammal or a tick.

19. The method of claim 10 wherein the presence of said Borrelia burgdorferi or said Borrelia burgdorferi exported antigens is indicative of Lyme disease caused by an infection of Borrelia burgdorferi in said mammal.

20. A diagnostic kit comprising:
A) purified antibodies or antigen binding fragments of said antibodies raised against the major extracellular protein antigen exported from Borrelia burgdorferi having a molecular weight of approximately 83 kDa, and

B) purified polyclonal antibodies or antigen-binding fragments of said polyclonal antibodies raised against the extracellular membrane vesicles exported from Borrelia burgdorferi.

21. The kit of claim 20, wherein the antibodies of element B) are bound to a solid substrate.

22. The kit of claim 21, wherein the antibodies of element A) are labeled with a detectable label.

23. The kit of claim 22, wherein the detectable label is chosen from the group consisting of radioisotopes, enzymes, fluorophores and colloidal metals.

24. The kit of claim 20 further comprising a specific binding protein which specifically binds to the antibodies of element A), said specific binding protein being labeled with a detectable label.

25. The kit of claim 24, wherein said specific binding protein is an antibody or protein A.

26. The kit of claim 25, wherein said detectable label is chosen from the group consisting of radioisotopes, enzymes, fluorophores or colloidal metals.

1/3,AB,CM/19 (Item 19 from file: 654)

02157586
Utility
METHOD AND MATERIALS FOR DETECTING LYME DISEASE
[Collecting a biological sample from a mammal, isolating immune complexes suspected of containing borrelia antibodies, dissociating complex to release antibody and determining the presence of antibodies by specific binding reaction]

PATENT NO.: 5,187,065
ISSUED: February 16, 1993 (19930216)
INVENTOR(s): Schutzer, Steven E., 21 Canterbury Rd., Great Neck, NY (New York), US (United States of America), 11021 68000]
APPL. NO.: 7-455,175
FILED: December 22, 1989 (19891222)

FULL TEXT: 1117 lines

ABSTRACT

A method for detecting the onset or presence of Lyme disease in a mammal, which comprises isolating a biological sample from the mammal, isolating from said biological sample any circulating immune complexes suspected to contain antibody reactive to Borrelia burgdorferi, dissociating the immune complexes so isolated, and examining the dissociated immune complexes for the presence of antibody. The present method offers a simple and reliable means for detecting Borrelia antibodies. Test kits and related methodology are also disclosed.

What is claimed is:

1. A method for detecting the onset or presence of Lyme disease caused by Borrelia burgdorferi infection in a mammal, which comprises:
A) collecting a biological sample from said mammal;
B) isolating from said sample any immune complexes suspected of containing antibodies to Borrelia burgdorferi;
C) dissociating any immune complexes isolated in step B) to release any of said antibodies to Borrelia burgdorferi present therein; and

E) determining the presence of said antibodies through a specific binding reaction as indication of Lyme disease in said mammal.

2. The method of claim 1 wherein said biological sample is selected from the group consisting of synovial fluid, pericardial fluid, serum, cerebrospinal fluid and tissue.

3. The method of claim 2 wherein said biological sample is serum.

4. The method of claim 2 wherein said biological sample is cerebrospinal fluid.

5. The method of claim 1 wherein said immune complexes are isolated by with polyethylene glycol precipitation and said immune complexes are dissociated by treatment with a solution including sodium borate.

6. The method of claim 1 wherein said specific binding reaction is an immunochromatographic assay.

7. The method of claim 1 wherein said specific binding reaction is an enzyme immunoassay.

8. The method of claim 1 wherein said specific binding reaction is a radioimmunometric assay.

9. The method of claim 1 wherein isolating said immune complexes is performed by incubation with Raji cells.

10. The method of claim 1 wherein determining the presence of said antibodies of step D further comprises:
i) immobilizing on a suitable solid support a specific binding partner directed to said antibodies to Borrelia burgdorferi;
ii) incubating said support, the antibodies released in step C) and detectably labeled antibodies to Borrelia burgdorferi, wherein the labeled antibodies compete with the antibodies released for specific binding with the immobilized specific binding partner;
iii) separating the material from step ii) which is bound to said support from the material not bound to said support; and
iv) determining the presence of said labeled antibodies bound to said support, whereby the presence of said labeled antibodies is indicative of the presence of Lyme disease.

11. The method of claim 1 wherein determining the presence of said antibodies of step D further comprises:
i) immobilizing on a suitable solid support antibodies to Borrelia burgdorferi;
ii) incubating said support, the antibodies released in step C) and detectably labeled specific binding partner directed to said antibodies to Borrelia burgdorferi wherein the antibodies released compete with the antibodies immobilized on said support for binding with the labeled specific binding partner;
iii) separating the material from step ii) which is bound to said support from the material not bound to said support; and
iv) determining the presence of the detectably labeled specific binding partner bound to said support, as an indication of the presence of Lyme disease.

12. The method of claim 1 wherein determining the presence of said antibodies of step D further comprises:
i) immobilizing on a suitable solid support a specific binding partner directed to said antibodies to Borrelia burgdorferi;
ii) incubating said support, the antibodies released in step C) and detectably labeled anti-species antibodies which bind to the antibodies to Borrelia burgdorferi for a time and under conditions sufficient to form a complex bound to said support;
iii) separating the material from step ii) which is bound to said support from the material not bound to said support; and
iv) determining the presence of said labeled anti-species antibodies bound

to said support, whereby the presence of said labeled anti-species antibodies is indicative of the presence of Lyme disease.

13. The method of claim 10, 11, or 12 wherein the label is selected from the group consisting of an enzyme, and two or more enzymes.

14. The method of claim 13 wherein the label is selected from the group consisting of peroxidase, beta -glucuronidase, beta -D-glucosidase, beta -D-galactosidase, urease, glucose oxidase plus peroxidase, galactose oxidase plus peroxidase, and alkaline phosphatase.

15. The method of claim 10, 11 or 12 wherein the label is a chemical which fluoresces when exposed to ultraviolet light.

16. The method of claim 15 wherein the chemical is selected from the group consisting of fluorescein, rhodamine, and auramine.

17. The method of claim 10, 11 or 12 wherein the label is a radioactive element.

18. The method of claim 17 wherein the radioactive element is selected from the group consisting of sup 14 C, sup 125 I, sup 131 I, sup 35 S, and sup 3 H.

19. A method for detecting Borrelia burgdorferi infection of a mammal which comprises:
A) collecting a biological sample from said mammal;
B) isolating from said sample any immune complexes suspected of containing Borrelia burgdorferi antigen;
C) dissociating any immune complexes isolated in step B) to release any of said Borrelia burgdorferi antigen present therein; and
E) determining the presence of said Borrelia burgdorferi antigen through a specific binding reaction as indication of Borrelia burgdorferi infection of said mammal.

1/3,AB,CM/20 (Item 20 from file: 654)

02122403
Utility
ASSAY FOR **LYME** DISEASE
[Using dye detection reagent]

PATENT NO.: 5,155,022
ISSUED: October 13, 1992 (19921013)
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[Assignee Code(s): 8488]
EXTRA INFO: Expired, effective October 16, 1996 (19961016), recorded in O.G. of December 24, 1996 (19961224)
APPL. NO.: 7-652,681
FILED: February 08, 1991 (19910208)
FULL TEXT: 524 lines

ABSTRACT

Diagnosis of Lyme disease by immunoassay for anti-Borrelia burgdorferi antibodies in the serum of a patient includes adsorption of crossreactive antibodies in the serum with antigen of Acinetobacter calcoaceticus or

Treponema phagedensis prior to binding of anti-Borrelia antibodies to Borrelia burgdorferi antigen absorbed onto a solid support. The preferred assay is a flow-through assay using a porous membrane as the support and a dye-loaded liposome conjugated to a goat antihuman antibody for detection. The invention includes a kit of materials for performing the assay.

What is claimed is:

1. A method of assay for anti-Borrelia burgdorferi antibodies in a serum sample comprising:
a) preparing a mixture of the serum sample and an Acinetobacter calcoaceticus absorbing antigen such that cross-reacting antibodies in the sample bind to the absorbing antigen;
b) passing the mixture through a porous membrane coated with a Borrelia burgdorferi capture antigen such that the anti-Borrelia burgdorferi antibodies bind to the capture antigen on the membrane and the cross-reacting antibodies bound to the absorbing antigen do not substantially bind to the capture antigen;
c) contacting the bound anti-Borrelia burgdorferi antibodies with a detection antibody conjugated to a liposome encapsulating an absorbing dye such that the detection antibody binds to the anti-Borrelia burgdorferi antibodies bound to the capture antigen; and
d) detecting the presence of the anti-Borrelia burgdorferi antibodies by detecting the dye on the membrane.

2. A method of assay for anti-Borrelia burgdorferi antibodies in a serum sample comprising:
a) preparing a mixture of the serum sample and an Acinetobacter calcoaceticus absorbing antigen such that cross-reacting antibodies in the sample bind to the absorbing antigen;
b) passing the mixture through a porous membrane coated with a Borrelia burgdorferi capture antigen such that the anti-Borrelia burgdorferi antibodies bind to the capture antigen on the membrane and the cross-reacting antibodies bound to the absorbing antigen do not substantially bind to the capture antigen;
c) contacting the bound anti-Borrelia burgdorferi antibodies with a detection antibody conjugated to a dye such that the detection antibody binds to the anti-Borrelia burgdorferi antibodies bound to the capture antigen, and;
d) detecting the presence of the anti-Borrelia burgdorferi antibodies by detecting the dye on the membrane.

3. A method of assay for anti-Borrelia burgdorferi antibodies in a serum sample comprising:
a) preparing a mixture of the serum sample and an Acinetobacter calcoaceticus absorbing antigen such that cross-reacting antibodies in the sample bind to the absorbing antigen;
b) contacting the mixture with a solid support coated with a Borrelia burgdorferi capture antigen such that the anti-Borrelia burgdorferi antibodies bind to the capture antigen on the solid support and the cross-reacting antibodies bound to the absorbing antigen do not substantially bind to the capture antigen;
c) contacting the bound anti-Borrelia burgdorferi antibodies with a detection antibody conjugated to a dye such that the detection antibody binds to the anti-Borrelia burgdorferi antibodies bound to the capture antigen; and;
d) detecting the presence of the anti-Borrelia burgdorferi antibodies by detecting the dye on the membrane.

4. A kit of materials for performing an assay for anti-Borrelia burgdorferi antibody in a serum sample comprising:
a) an enclosure;
b) a filter stack in the enclosure, the filter stack including a porous membrane and a pad of absorbent material in contact with the membrane, the membrane having thereon a coating of Borrelia burgdorferi capture antigen;
c) an Acinetobacter calcoaceticus absorbing antigen for absorbing cross-reacting antibodies in the serum sample, and;
d) a tracer comprising a detection antibody labeled with a dye.

5. A kit of materials for performing an assay for anti-Borrelia burgdorferi antibody in a serum sample comprising:
a) an enclosure;
b) a filter stack in the enclosure, the filter stack including a porous membrane and a pad of absorbent material in contact with the membrane, the membrane having thereon a coating of Borrelia burgdorferi capture antigen;
c) an Acinetobacter calcoaceticus absorbing antigen for absorbing cross-reacting antibodies in the serum sample, and;
d) a tracer comprising an antihuman detection antibody conjugated to a liposome encapsulating an absorbing dye.

6. The method according to claims 1, 2 or 3 wherein the serum sample is mixed with an absorbing antigen comprising intact microbial cells.

7. The method according to claims 1, 2 or 3 wherein the serum sample is mixed with an absorbing antigen comprising a sonicate of microbial cells.

8. The method according to claims 1, 2 or 3 wherein the bound antibodies are contacted with a detection antibody comprising an anti-human antibody.

9. The method according to claims 2 or 3 wherein the boudn antibodies are contacted with a detection antibody conjugated to a dye selected from the group consisting of fluorescent dyes and absorbing dyes.

10. The method according to claim 1 wherein the bound antibodies are contacted with a detection antibody conjugated to a liposome encapsulating a dye selected from the group consisting of fluorescent dyes and absorbing dyes.

11. The method according to claim 2 wherein the membrane is further coated with an inert protein.

12. The kit according to claim 4 or 5 wherein the absorbing antigen comprises intact microbial cells.

13. The kit according to cliam 4 or 5 wherein the absorbing antigen comprises a sonicate of microbial cells.

14. The kit according to claim 4 or 5 wherein the detection antibody is an anti-human antibody.

15. The kit according to claim 4 or 5 wherein the dye is selected from the group consisting of fluorescent dyes and absorbing dyes.

1/3,AB,CM/21 (Item 21 from file: 654)
DIALOG(R)File 654:US PAT.FULL.

02105789
Utility
SUBSTRATE COMPOSITION AND METHOD FOR SOLID PHASE UREASE IMMUNOASSAY
[Colored detectable product which precipitates on solid support]

PATENT NO.: 5,139,934
ISSUED: August 18, 1992 (19920818)
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[Assignee Code(s): 8488]
EXTRA INFO: Expired, effective August 21, 1996 (19960821), recorded in O.G. of October 29, 1996 (19961029)

APPL. NO.: 7-528,526
FILED: May 25, 1990 (19900525)
FULL TEXT: 410 lines

ABSTRACT

A method for enzyme immunoassay of a ligand includes urease as a label. A bound fraction of ligand and antiligand conjugated to urease is formed on a solid support. The urease component of the bound fraction is contacted with a substrate composition for urease which includes a compound converted to ammonia by the urease, a tetrazolium salt and a pH dependent reducing agent which reduces the tetrazolium salt when the pH of the assay medium has been raised by the ammonia. The tetrazolium salt is reduced to a colored insoluble formazan which precipitates as a detectable spot on the support. The invention includes the substrate composition and a kit of materials for performing the assay.

What is claimed is:

1. A method for detecting a ligand in a liquid comprising:
a) passing a liquid suspected of containing said ligand through a porous membrane precoated with a capture antiligand such that said ligand is captured on said membrane;
b) passing through said membrane a tracer solution comprising urease conjugated to a molecule selected from the group consisting of the ligand and a detection antiligand such that said urease becomes affixed to said membrane; and
c) passing through said membrane a solution comprising (i) a compound converted by said urease to ammonia, (ii) a pH-dependent reducing agent selected from the group consisting of ascorbic acid and indoxyl and (iii) a tetrazolium salt, said urease affixed to the membrane converting said compound to ammonia, said ammonia raising the pH of the solution thereby activating said reducing agent, the activated reducing agent reducing said tetrazolium salt to a formazan, said formazan precipitating as a colored spot on said membrane and indicating the presence of the ligand in said liquid.

2. The method of claim 1 wherein said ligand is selected from the group consisting of an antigen, an antibody and a hapten.

3. The method of claim 2 wherein said ligand is an antigen or a hapten and said capture antiligand is an antibody specific for said antigen or said hapten.

4. The method of claim 2 wherein said ligand is an antibody and said capture antiligand is an antigen specific for said antibody.

5. The method of claim 1 wherein said tracer comprises urease conjugated to the ligand, and said ligand and said tracer compete for binding sites on said capture antiligand.

6. The method of claim 1 wherein said tracer comprises urease conjugated to said detection antiligand and said tracer to said ligand.

7. The method of claim 1 wherein said compound is selected from the group consisting of urea, N-methyl urea, semicarbazide, formamide and acetamide.

8. The method of claim 1 wherein the precoated membrane is prepared by coating with said capture antiligand and coating with an inert protein to block unfilled binding sites on the precoated membrane.

9. The method of claim 8 wherein said inert protein is selected from the group consisting of albumin and casein.

10. The method of claim 1 wherein said tetrazolium salt is selected from the group consisting of iodonitrotetrazolium violet and nitrobluetetrazolium chloride.

11. A method for detecting a ligand in a liquid comprising:
a) contacting a liquid suspected of containing said ligand with a solid support such that said ligand attaches to said support;
b) contacting the ligand attached to the support with a tracer comprising urease conjugated to an antiligand such that said antiligand binds to the attached ligand and said urease becomes affixed to said support; and
c) contacting the urease affixed to said support with a substrate composition for said urease, said composition comprising a compound converted by said urease to ammonia, a tetrazolium salt and a pH-dependent reducing agent for said tetrazolium salt selected from the group consisting of ascorbic acid and indoxyl whereby production of ammonia by said urease causes a rise in pH sufficient to activate the reducing agent such that the tetrazolium salt is reduced by said activated reducing agent to a colored formazan which forms a colored spot on said support of said ligand is present in said liquid.

12. The method of claim 11 wherein said support is a dipstick.

13. The method of claim 11 wherein said support is a porous membrane.

14. The method of claim 11 wherein said support is an immunochromatography plate.

15. A method for detecting an antigen in a liquid comprising:
a) combining a liquid suspected of containing the antigen with a porous membrane coated with a capture antibody and with a detection antibody conjugated to urease such that said antigen binds to said capture and detection antibodies to give a membrane bound fraction comprising said urease on said membrane;
b) allowing said liquid to pass through said membrane; and
c) passing a substrate composition comprising urea, ascorbic acid and a tetrazolium salt through said membrane, said urease bound on the membrane converting said urea to ammonia, said ammonia activating said ascorbic acid to reduce said tetrazolium salt to a formazan, said formazan precipitating as a colored spot on said membrane and indicating the presence of said ligand in the liquid.

16. The method of claim 15 wherein the coated membrane is prepared by coating with said capture antibody and coating with an inert protein to block unfilled binding sites on the coated membrane.